THE SIR WILLIAM MACLEAY MEMORIAL LECTURE 1987

The elastic-sided Gumleaf, or: The Rubber Cuticle and other Studies of the *Corymbosae*

STELLA G. M. CARR and D. J. CARR

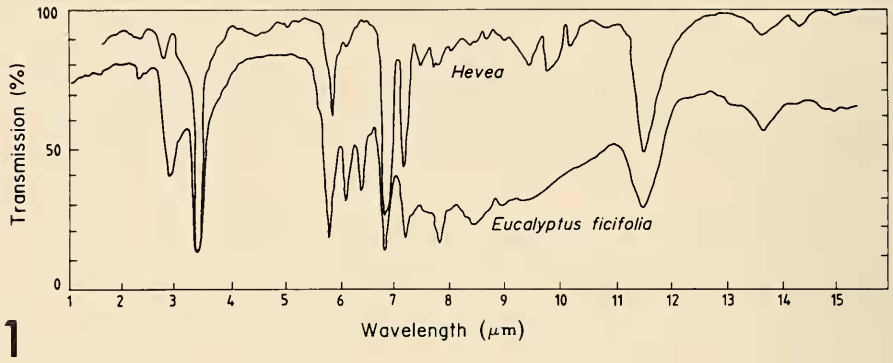
Research School of Biological Sciences,
Australian National University, Canberra

[Delivered by D. J. CARR 30 September 1987]

Introduction: the rubber cuticle of the *Corymbosae*

In 1908, the Sydney phytochemist Henry G. Smith, of the Technological Museum, published an account of his analysis of the cuticles of *Eucalyptus gummifera* and those of two species of *Angophora*. He showed that, using suitable solvents, a material could be extracted from young leaves of these species which had chemical and physical properties identical with those of natural rubber (caoutchouc). It was soluble in chloroform, and in absolute ether but not in petroleum ether. Various tests, including melting point and ability to be vulcanized, convinced Smith that the material was indeed rubber. It has long been known that the cuticles of young leaves of species like *E. gummifera*, spotted gum (*E. maculata*), and red flowering gum (*E. ficifolia*), are easily removed almost intact from the leaf surfaces and have the elastic properties of rubber. Later on, as the leaves mature and become fully developed, the cuticle undergoes changes which prevent it from being stripped off the leaves. This extraordinary discovery has not rated mention in any of the botanical textbooks, in the many reviews of recent decades on the topic of the plant cuticle or in two monographs devoted to that subject (Martin and Juniper, 1970; Cutler *et al.*, 1982). The only reference we have been able to find is in the 2nd Edition of Metcalfe and Chalk (an expensive, poor relation of the magnificent first edition), in which Metcalfe (1983) reported that 'the secretion of a rubbery substance from epidermal papillae on young shoots of three species of *Angophora* and fourteen species of *Eucalyptus* belonging to the section *Corymbosae* (Myrtaceae) has been reported by Welch (1923)'. Metcalfe thus added confusion to the already confused views of Welch, who in describing the papillate epidermis of the *Corymbosae* as a secretory tissue, ignored the fact that all shoot epidermes which secrete a cuticular layer are also, *ipso facto*, secretory tissues, whether papillate or not. Welch was somewhat misled by the fact that during embedding in paraffin the rubber tends to dissolve in the embedding medium and be at least partially lost. Metcalfe failed to follow up the reference to Smith's work given by Welch. Moreover one of Welch's 14 eucalypts is '*E. santalifolia*', which, as he points out, does *not* belong to the *Corymbosae*. Nor, as Blake (1953) later pointed out, should *E. tessellaris*, another of Welch's species, be included in the *Corymbosae*. Welch did, at least, recognize the material covering the leaves as a *cuticle* and not, to use Metcalfe's phrase, mere 'rubbery secretions'. In his article on 'Secretory Structures: cells, cavities and canals', Metcalfe appears to have followed Nelsonian principles in turning a blind eye to much of the recent literature.

In 1956, when we commenced our studies of the biology of the eucalypts, we repeated Smith's extractions and most of his tests, using young developing leaves of *E. ficifolia* grown in cultivation in Melbourne. We were not only able to verify Smith's



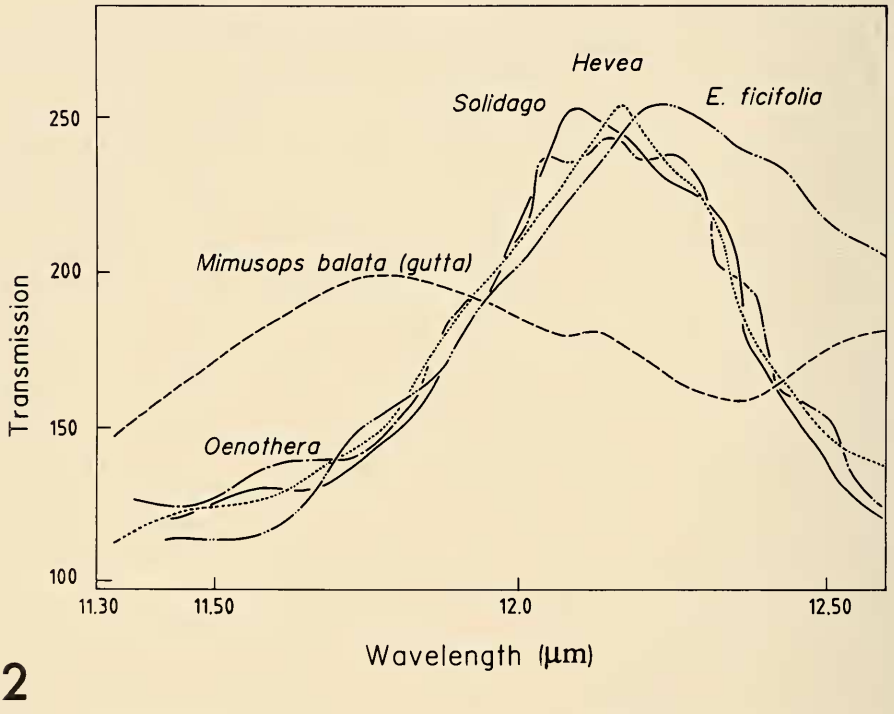


Fig. 1. Infra-red transmission spectrograms; abscissa, microns. **1.** Rubber from *Hevea brasiliensis* and a cuticle preparation from *Eucalyptus ficifolia.* **2.** Comparisons between rubber from various sources, gutta percha, and a cuticle preparation from *Eucalyptus ficifolia.* (Data courtesy Dr T. P. O'Brien and the late Sterling B. Hendricks).

claims, but we also were able to produce little balls of rubber with a very satisfactory bounce. The most favoured method for the identification of rubber is infra-red absorption spectroscopy. Dr T. P. O'Brien, a former student of ours, then employed at ICI in Melbourne, ran some IR absorption spectra of whole cuticles and arranged to send them for comment to Sterling B. Hendricks at the Beltsville Laboratory of the US Department of Agriculture (Fig. 1). Dr Hendricks, by training a physicist and a spectro-

scopist who had worked with Linus Pauling, had been involved during the last World War in a programme of investigation of various plants (e.g. guayule and the kok-saghyz dandelion) as sources of natural rubber. He examined the IR spectra and in reply to Dr O'Brien stated that 'your samples appear to me on the basis of absorption in the region of 12 microns' (a critical region) 'to be rubber'. More recently we repeated the extraction of rubber from young leaves of *E. calophylla* sent to us by Mrs M. J. Hamersley from Western Australia. Using the samples thus obtained we carried out the chemical test known as Weber's test for rubber. A small sample, 50mg, is cut into fine pieces and put into a test tube. A drop of bromine is added and the test tube is warmed for 30 seconds on a water bath. Then a gram of solid phenol is added. Natural rubber gives a violet coloration, but synthetic rubbers, such as neoprene, give only a weak reaction. Needless to say, the rubber samples from *E. calophylla* gave a strong positive reaction.

The two eucalypt species mentioned so far belong to a group commonly called bloodwoods, or botanically the *Corymbosae*. Following Smith's publication, Maiden (1908-1928) and Welch (1923) claimed to have observed rubber in a variety of species of eucalypts in other taxonomic groups. We have no evidence on this point, although it is inherently not unlikely.

The rubber cuticle is layered

In a number of ways, the cuticle of the *Corymbosae* is different from that of other angiosperms. The consensus of opinion appears to be that, in angiosperms generally, the cuticle steadily increases in thickness as the leaf or stem grows. In the *Corymbosae* the cuticle of the leaf increases in thickness only until the leaf is between a third and a half fully-grown. It then decreases in thickness until near maturity (Fig. 2). The parameters on which these graphs were constructed are discussed in our recent book, *Eucalyptus II*. We would emphasize that much more information (data on plastochrone duration, measurements of the duration and extent of lamina growth, measurements of average cuticle thickness during growth) is needed to establish and quantify these concepts but there can be no doubt that the cuticle thickness does increase and then decrease. This can be seen from a hand-section of a leafy bud of a species such as *E. gummifera*. Clearly then, the rubber cuticle is laid down at an early stage on the surface of the leaf primordium and is subsequently stretched and thins out during what used to be called the 'grand phase of growth' of the leaf. The cuticle remains thick over the midrib and margins of the leaf which expand mainly in length and thickness, especially on the lower surface, (thus increasing its area relative to the upper surface) during the later phases of leaf growth. Welch measured the thickness of the cuticle on a leaf of *E. gummifera* less than 1 millimetre wide to be between 170 and 185μm. Our own measurements of *E. maculata* grown in Canberra show a much more modest maximum thickness. Since the leaves appear in pairs in the buds, with the inner (adaxial) faces of each pair adherent, the thickness of the cuticle on those surfaces lags behind that of the freer abaxial surfaces. Later on, the thickness of the cuticle on the upper surface catches up and at maturity is thicker than that of the lower surface. This is due to the fact that, at a late stage in leaf expansion, the epidermal cells begin to lay down layers of cutinized cuticle (Fig. 3). These develop to a greater thickness on the upper surface than on the lower. They are also thicker on the midrib and margin than elsewhere. Stomata are initiated and begin to break through to the outer surface of the cuticle at or just before the time the leaf is half fully-grown, while the cuticle is beginning to be stretched and thin out. All the stomata are already formed by the time the cuticularized layers begin to be laid down. We shall return to the stomata later on. The flower bud and its constituent parts, such as the sepals, petals, style, stamens and the loculi of the ovary are also covered with

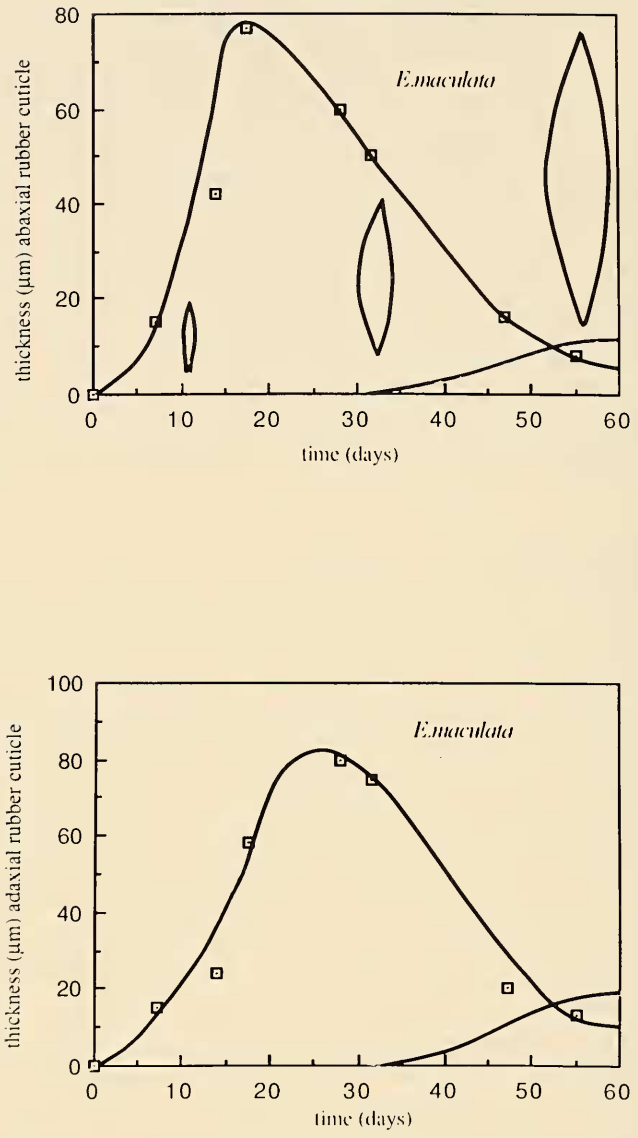


Fig. 2. Graphs to show changes in the thickness of the abaxial (upper graph) and adaxial cuticle of an adult leaf of *E. maculata*. The lines on the lower right of each graph beginning after c.32 days, show the formation of the cuticularized layers underlying the rubber cuticle.

a rubber cuticle, which is often massively thick, and, at maturity, is not underlain by cuticularized layers.

By suitable staining one can demonstrate that the whole of the thick rubber cuticle consists of layers, each consisting of a thin dark layer and a thicker lightly-staining one (Fig. 4). It is often possible to count the layers in a section of the leaf and in a leaf of *E. maculata* estimated to be between 30 and 40 days old, when the cuticle of the adaxial surface is at its maximum thickness, the number of layers is between 30 and 40. This is

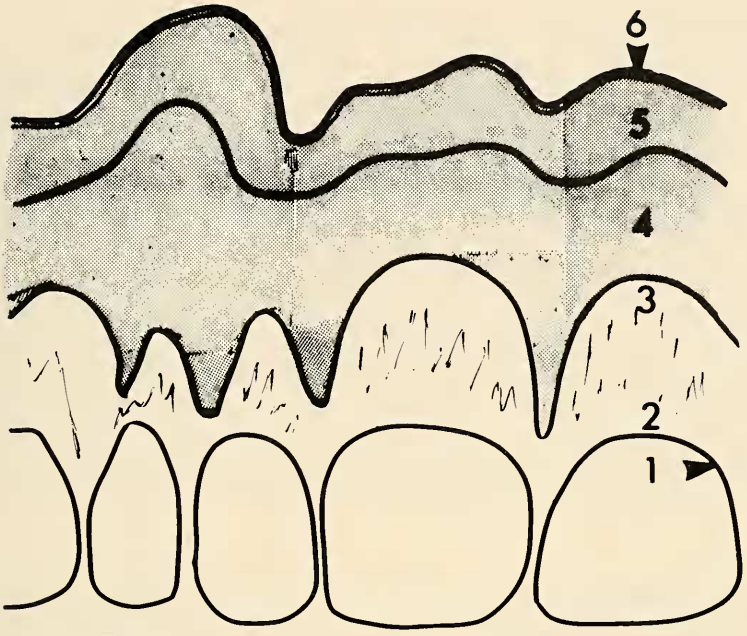


Fig. 3. Diagram to show zonation in the cuticle of a mature, fully-grown leaf of a species of *Corymbosae*. 1, cell wall. 2, 3 cuticularized layer, layer 3 with radial striae. 4, 5 zones of rubber cuticle, the layers well-spaced in 4, closely-spaced in 5. 6, the 'cuticle proper'.

at least presumptive evidence that each bilayer is the product of a single day's secretory activity on the part of the epidermis. A similar diurnal layering is well-known for the cell-walls of certain algae and for the hairs on the seeds of cotton. Moreover, the cuticles of insects have diurnally-produced layers (Neville, 1963). The difference in density between the two portions of a bilayer may reflect different packing densities of the rubber in them or perhaps differences in ancillary materials associated with the layers. Again, we wish to stress that much more work is needed to establish the diurnal rhythm in rubber production, the nature of the differences in the bilayers, the nature of ancillary materials in the rubber cuticle etc.

Stomatal breakthrough in the *Corymbosae*

As we have already shown in studies of the stomatal development in other groups of eucalypts, the stomatal initials appear and the full structure of the guard cells develops, including the split between them, while the guard cells are completely covered by an unbroken cuticle (Fig. 5). Above the line of closure of the guard cells a split must develop in the cuticle to give access to the atmosphere. We have provided evidence from light microscopy which supports the view that this split, resulting in *stomatal breakthrough*, develops by a process of digestion of the cuticle above the guard cells, probably by enzymes secreted by them into the cuticle (Carr and Carr, 1978; Carr and Carr, in preparation). The hole in the outer surface of the cuticle which leads to the guard cells we termed the *ostiole* (Carr and Carr, 1978). Although an essential part of the functional stoma, and, in thick cuticles, often smaller in area than the pore between the guard cells of the open stoma, it had not previously been given a name. Stace (1965) ignores it; Wilkinson (1979) calls it 'the outer stomatal ledge aperture', a long-winded appellation

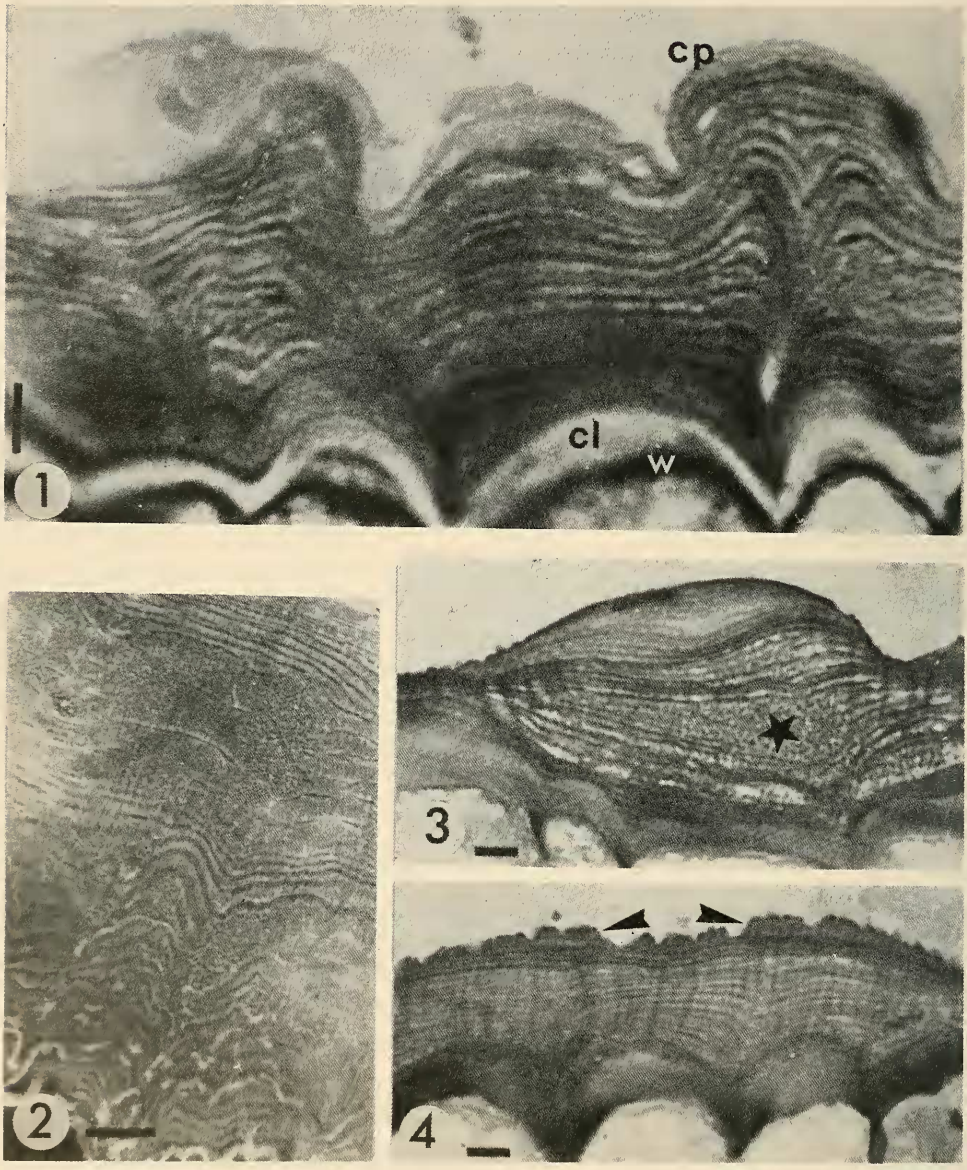


Fig. 4. Lamellate rubber cuticle of developing leaves of *E. maculata*. **1**, **3**, **4** adaxial surface, **2** abaxial surface. **2** leaf about half fully expanded; **3**, **4** fully expanded. In **2**, the number of bilayers (a light zone plus a dark line) is between 30 and 40. In **3** the asterisk indicates a widening of one of the light zones. In **4** arrows indicate positions where losses of outer layers can be observed. cp, cuticle proper; cl, cuticularized layer just beginning to be formed; w, cell wall. Scale bars, 10μm.

which is wrong for sunken stomata (e.g. those of *Proteaceae* spp., *Aloe* spp., *Ficus* spp., *Eucalyptus incrassata* (Carr and Carr, 1978), species of the *Bisectae* (*Eucalyptus*) and of the *Lehmannianae* (*Eucalyptus*) (Carr and Carr, 1980c), which have outer stomatal ledges which are not fused with the cuticle. The correct term for the aperture between the outer stomatal ledges is the *eisodial aperture*. The problems of stomatal breakthrough in the

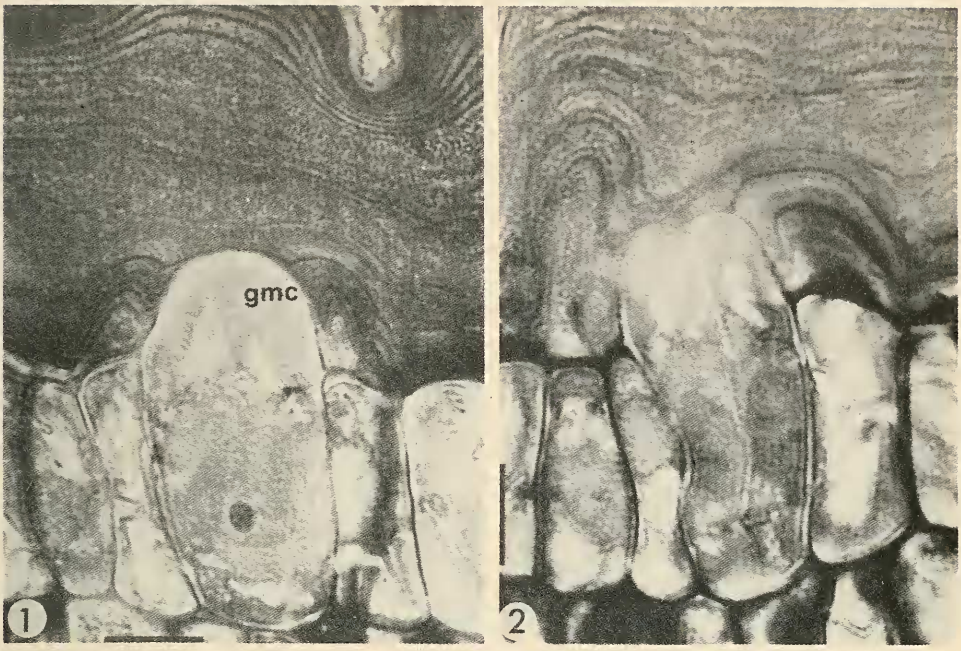


Fig. 5. Stomatal breakthrough, giant stomata. Developing leaf of *E. maculata*, abaxial surface. In **2** the guard mother cell (gmc) has divided, giving rise to the guard cell initials. Scale bar, 10μm.

Corymbosae are quite different from those we have observed in other eucalypts, or in common mesophytic plants such as *Phaseolus* and sunflower (Carr and Carr, in preparation), and are solved in quite a different manner, because of the presence of a layered, rubber cuticle above the stomatal complexes.

In the *Corymbosae*, the guard mother cell (GMC) expands and produces a vacuolate apical swelling or papilla which extends into the cuticle above it. As it touches the layers of rubber they appear to dissolve and their cut edges adhere to it (Fig. 5 [**1**]). The GMC divides anticlinally to yield the two guard cell initials (Fig. 5 [**2**]). Next the apical swelling relaxes so that the outer surface of the developing guard cells resumes a position level with the rest of the epidermal cells (Fig. 6 [**1**]). Still within the mother cell envelope, the two guard cells begin to lay down the layers of their upper and lower wall thickenings. These become cutinized (Fig. 6 [**2**]). They will eventually form the inner and (part of) the outer stomatal ledges. At this stage, above the developing guard cells a conical zone appears in the cuticle (Fig. 7). The zone is filled with a granular precipitate. It is evidently a region in which dissolution of the rubber layers takes place, the precipitate being, presumably, a product of the digestion of the rubber. A similar conical zone of dissolution has been reported by us as appearing above the developing guard cells of *Eucalyptus incrassata*, which has a thick *cutinized* (i.e. non-rubber) cuticle (Fig. 7 [**2**]). It is therefore timely to give such a zone a name; we have called it the *conus*, short for *conus dissolutionis*. Our interpretation of the origin of the conus is that the guard cells, still enclosed within their mother cell envelope, secrete into the cuticle enzymes capable of digesting the cuticle, whether it consists of rubber (as in the *Corymbosae*) or of cutinized layers. Very probably the GMC itself has some of the necessary enzyme (we may call it a

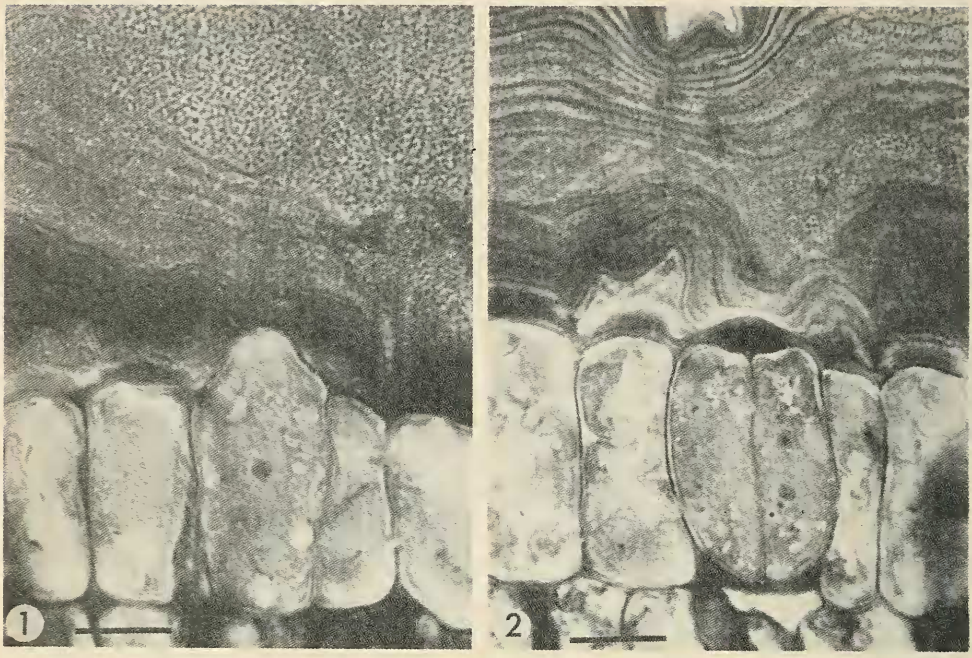


Fig. 6. As Fig. 5, to show later events in stomatal breakthrough. In **1** the guard cell initials have withdrawn dragging with them the innermost rubber lamellae. **2**, upper and lower guard cell thickenings have begun to form inside the old gmc envelope.

'*laticase*' in the *Corymbosae*) on its outer surface and that this is the reason for its ability to break through and attach to itself the rubber layers it touches.

As the conus enlarges and the number of still undigested rubber layers above it decreases, it begins to intersect with the outermost layers of rubber. At this stage, scanning electron microscopy of the leaf surface (Fig. 8 [**1**]) shows a number of black annuli (black because the thin cuticle is not as electron-reflective as elsewhere). Within such an annulus a circular line of breakage appears and the inner disc of rubber is released, shrivels and is lost (Fig. 8 [**2**]; Fig. 10 [**4,5,6**]). A more-or-less circular opening is left in the cuticle, leading down, through similar jagged holes in successive layers, to the guard cells. Inverted cuticles show these layers and the jagged edges of the holes made in them Fig. 8 [**6**]). This method of stomatal breakthrough applies to the earliest stomata formed on the leaf. These stomata, because they have an unusually high complement of subsidiary cells, are referred to as *giant stomata*. We shall have more to say about them in the second part of this lecture.

Stomata formed later on, when the cuticle is rapidly thinning, also each have a conus but it becomes stretched out by the lateral expansion of the cells of the stomatal complex and is shallow (Fig. 9 [**2**]). Where it intersects with the surface layers of the cuticle it presents more the appearance of a small dot or a line (Fig. 11 [**3,4,5**]). Cracks appear along such a line and join up to form a slit. No disc of rubber is released. As leaf expansion continues, the slit widens to become an ellipse (Fig. 11 [**8,9**]), often still showing at its ends the traces of the line which initiated it. Inverted cuticles show the shallow cavities in the cuticle, above the ordinary stomata (Fig. 8 [**5**]). Breakthrough in these ordinary stomata brings the outer thickenings of the guard cells in contact with the outermost layers of rubber (Fig. 9 [**4**]) and they fuse with them to form a composite *outer*

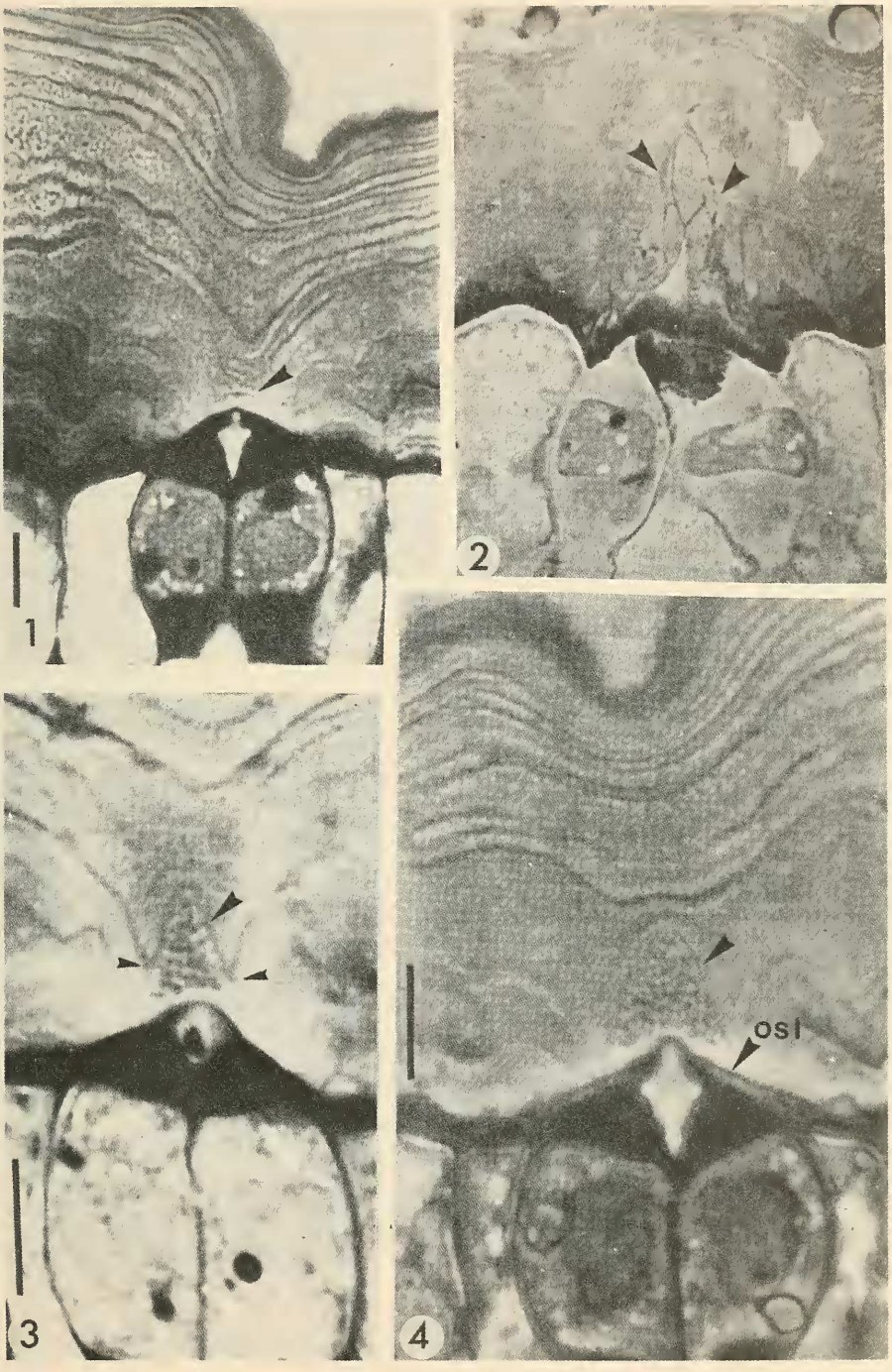


Fig. 7. All except **2** *E. maculata*, to show the initiation of the *conus dissolutionis* (arrowheads) outside the gmc envelope. osl, outer stomatal ledge. **2**. The conus (arrows) above a developing stoma of *Eucalyptus incrassata*. White arrowhead (2), layering of the cuticularized cuticle. Scale bars, 10μ.

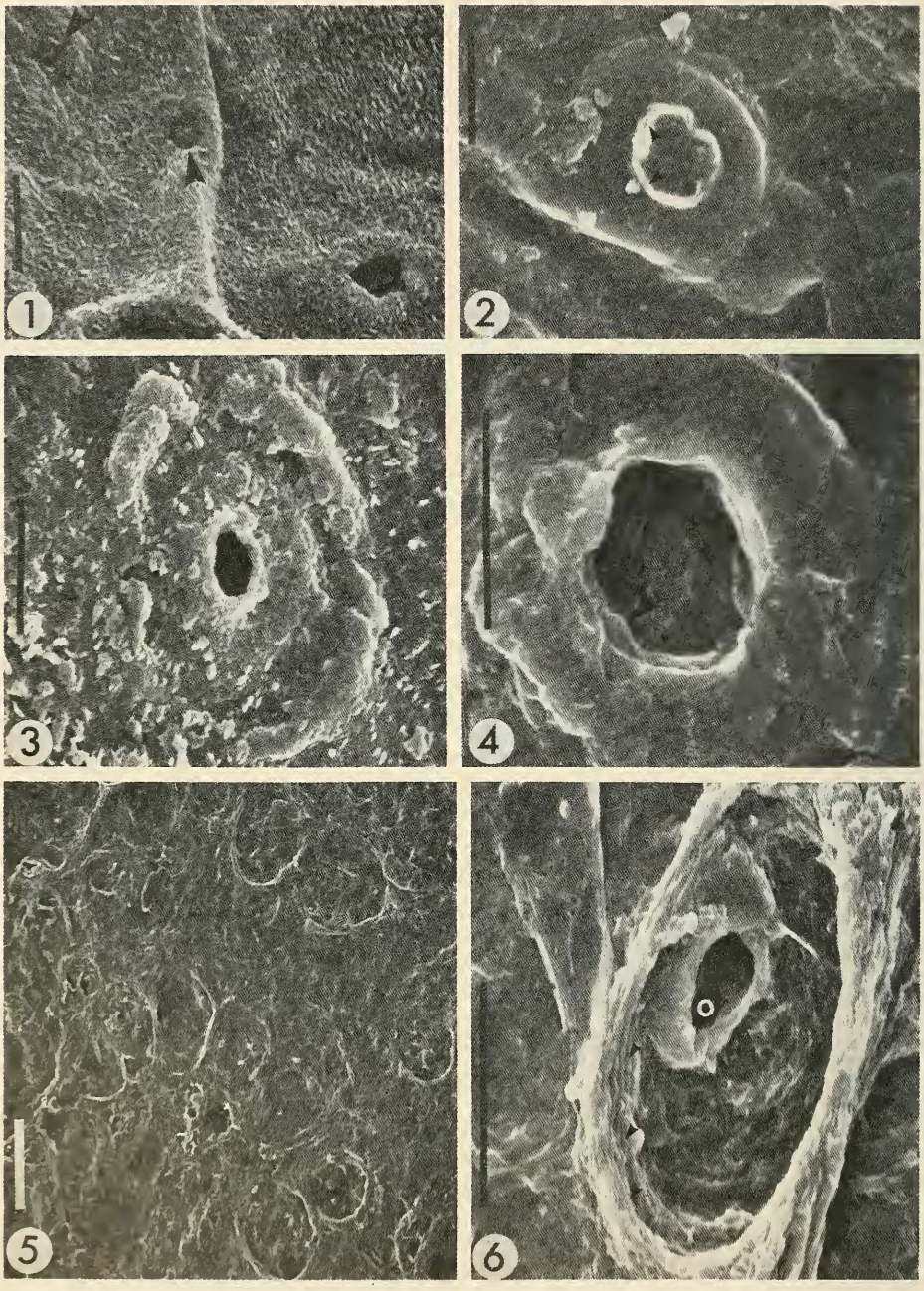


Fig. 8. Stomatal breakthrough, scanning electronmicrographs *E. erythrophloia*. **1**. Arrows, dark circles indicating imminent breakthrough. **2**. Detachment of the rubber disk, split appearing between two thickened rims of the annulus. **3**. Breakthrough completed and peristomatal cuticular ornamentation beginning to form. **4**. Through the ostiole, lamellae of the cuticle can be seen. **5** and **6**. Detached, inverted cuticle taken from a leaf at a late stage in stoma formation. Cup-shaped hollows in the cuticle where stomatal breakthrough has occurred or is occurring. **6**. One such area enlarged. Small arrowheads indicate lamellae in the cuticle. o, ostiole. Scale bars, all 10μm.

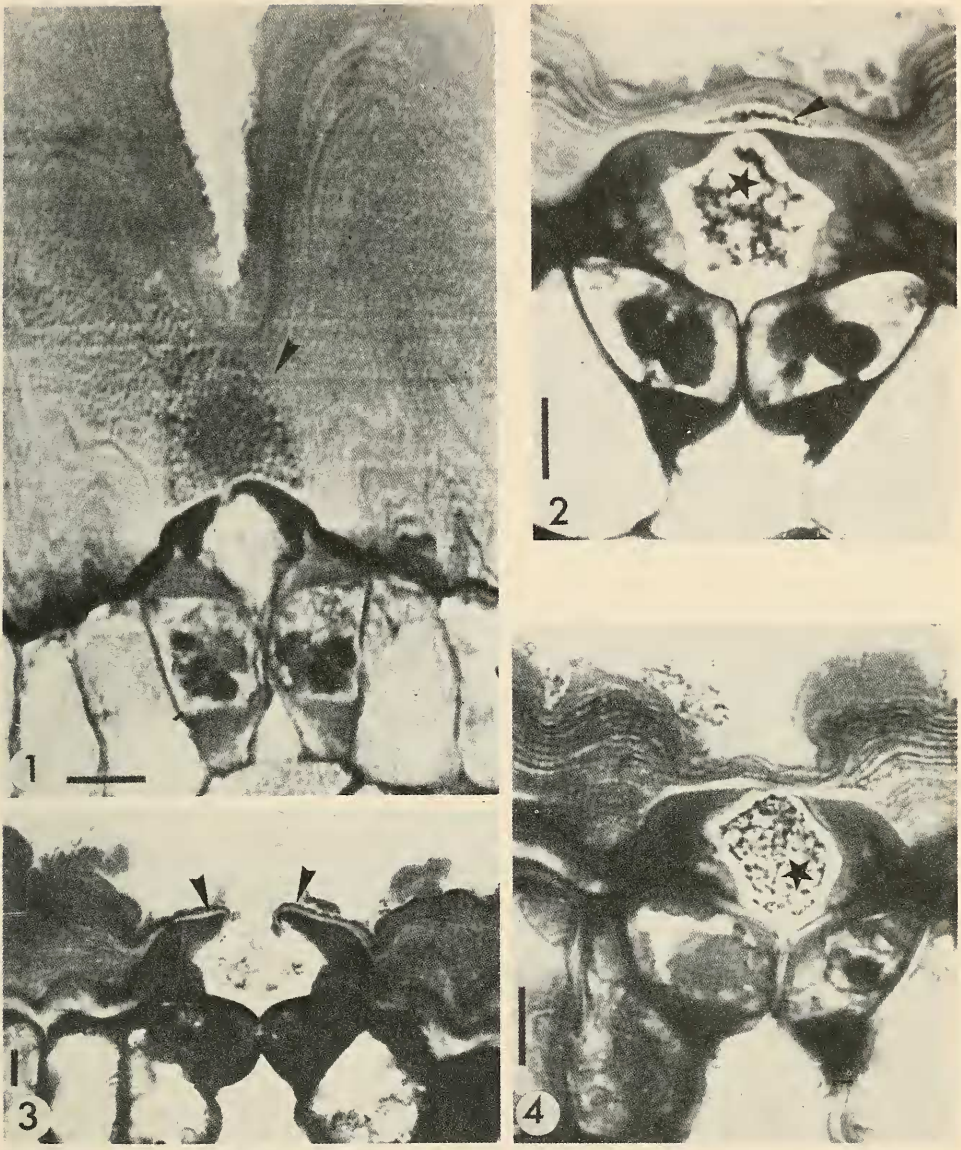


Fig. 9. E. maculata, completion of stomatal breakthrough. **1**. Large conus (arrow) of 'giant' stoma touching the outer surface of the cuticle. **2**. Small, flattened conus of 'ordinary' stoma. Guard cells almost fully formed. **3**. Completed ordinary stoma. Outer stomatal ledges formed as a composite of the guard cell thickenings and the outermost rubber layers. **4**. 'Ordinary' stoma about to break through to surface. Asterisks in **2** and **4** indicate accumulation in anterior chamber of stoma of breakdown products of the dissolution of rubber lamellae. Scale bars, all 10μm.

stomatal ledge (Fig. 9 [3]). Occasionally there is a developmental hiatus, in which the final breakthrough does not take place (Fig. 11 [7]). The stomata, although otherwise fully-formed, are 'blind', i.e. have no opening or *ostiole* on the surface, and are therefore clearly non-functional.

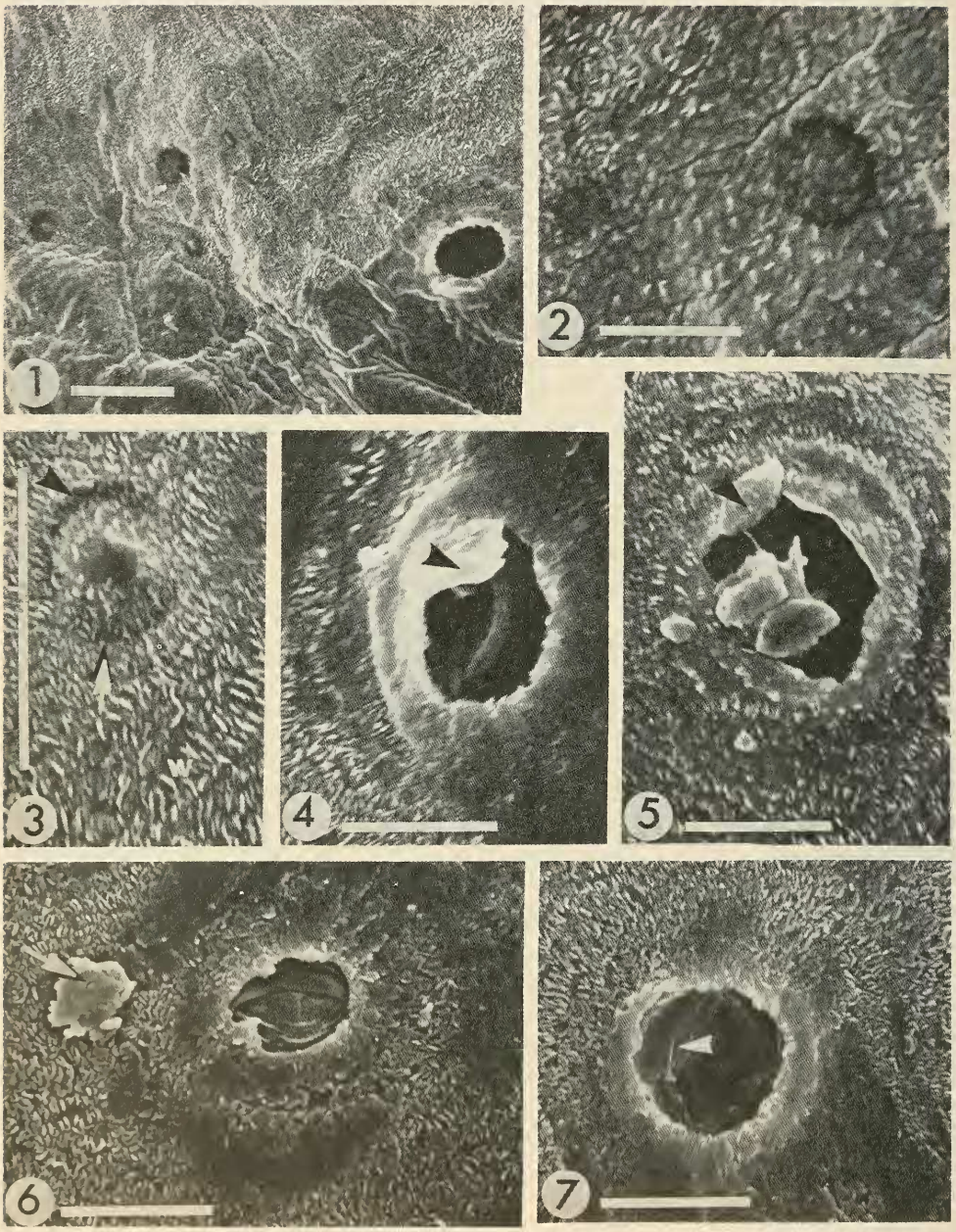


Fig. 10. Stomatal breakthrough, scanning electronmicrographs. *E. calophylla.* **1** and **2** dark rings, surrounding circular disks. **3**. Arrows indicate splitting of the circular disk from the rest of the (wax-covered) surface. **4**, **5**. Arrows indicate shrivelled disks still attached to the ostiole or **6** loose on the surface of the cuticle. **7**. Completed ostiole, through which one can observe (arrow) some of the lamellae of the cuticle. Scale bars, **1** 10μm; all to same magnification.

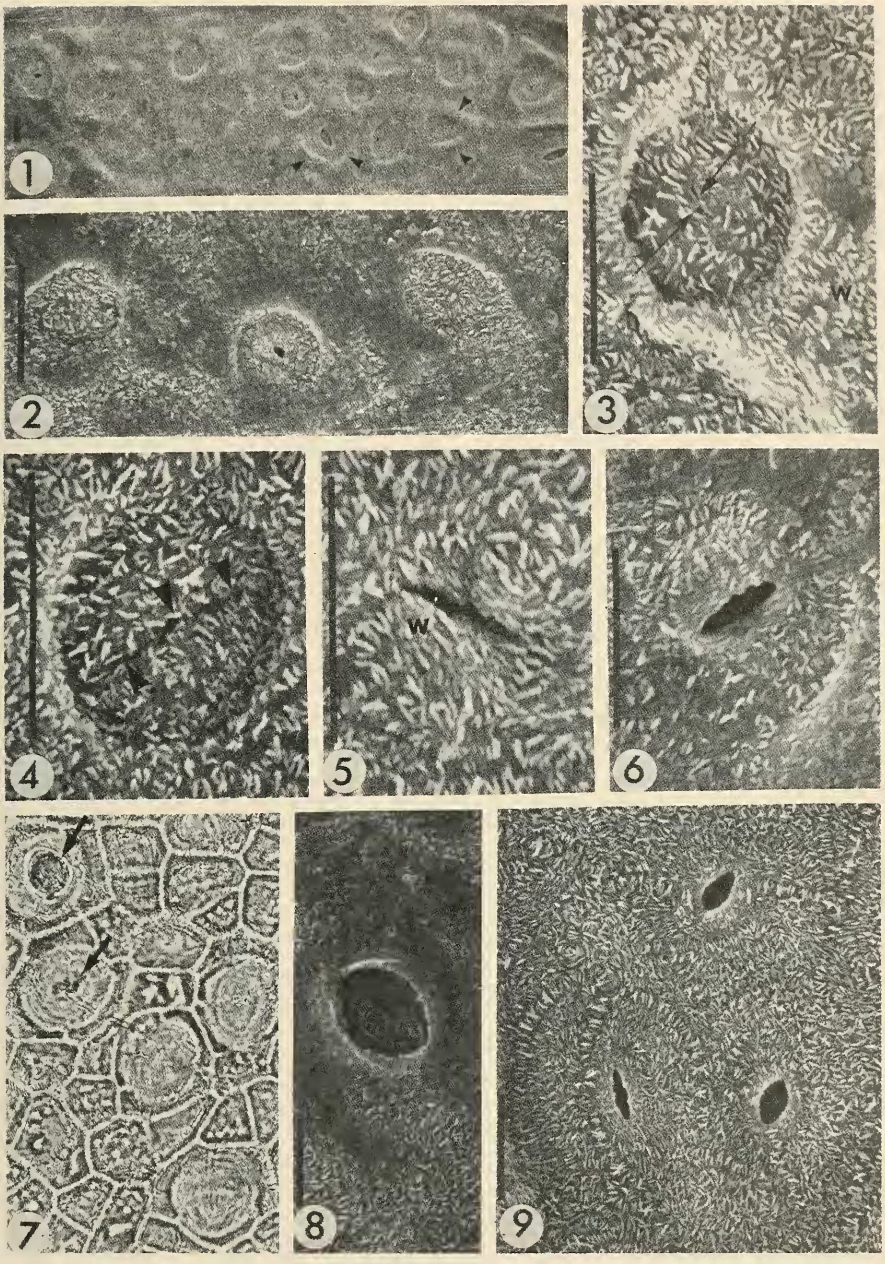


Fig. 11. Stomatal breakthrough of 'ordinary' stomata. Scanning electronmicrographs. **1-8**, *E. calophylla*, abaxial surface. **1** and **2**. A field of stomata developing and breaking through. Note that the peristomatal cuticular ornamentation has begun to form (arrowheads). **3-6**. Breakthrough beginning as a slit or crack (arrows) in the outer surface and **6** the ostiole completed. W, wax crystallites. **7**. Light micrograph of leaf surface of a specimen of *E. hamersleyana*, showing (arrows) stomata with ostioles; the other stomata have no ostioles and are non-functional ('blind'). **8**. *E. calophylla*. After breakthrough, the ostiole enlarges and becomes elliptical. In **8** the outer stomatal ledges are visible through the ostiole. **9**. *E. eremaea*. Fully-formed 'ordinary' stomata. Scale bars, all 10μm.

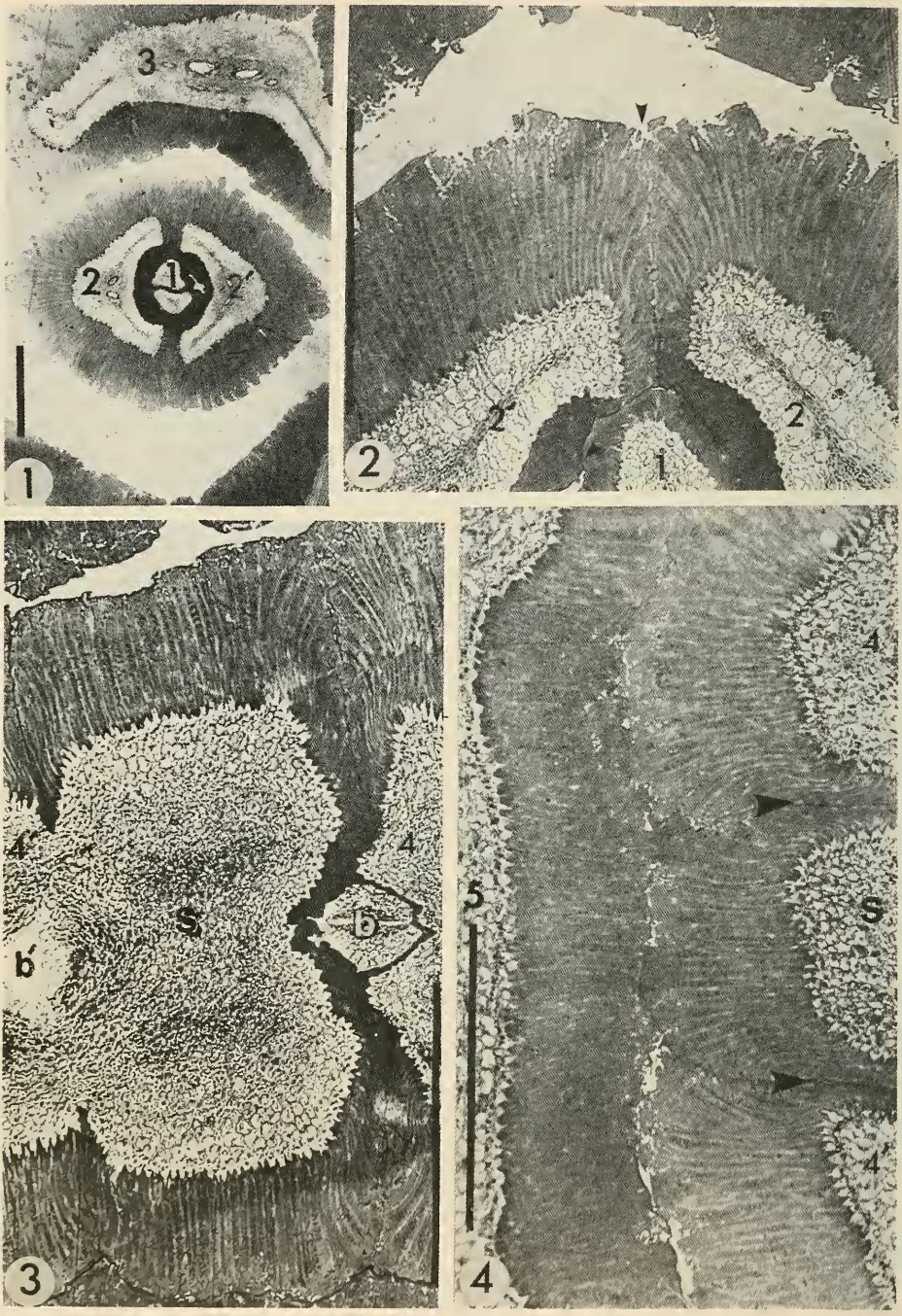


Fig. 12. Transverse sections of a leafy bud of *E. collina*. **1-5** transverse sections, **1** and **2** near the tip of the bud, **3** and **4** near its base. The leaf primordia are numbered in basipetal sequence, the precocious primordium of a pair with a prime (as e.g. 2′). b, b′, axillary buds (or, in b′, a position above an axillary bud), S, stem. Arrowheads indicate positions where the cuticle of one primordium abuts on that of another. Scale bars, **1** 1mm, all the others 10μm.

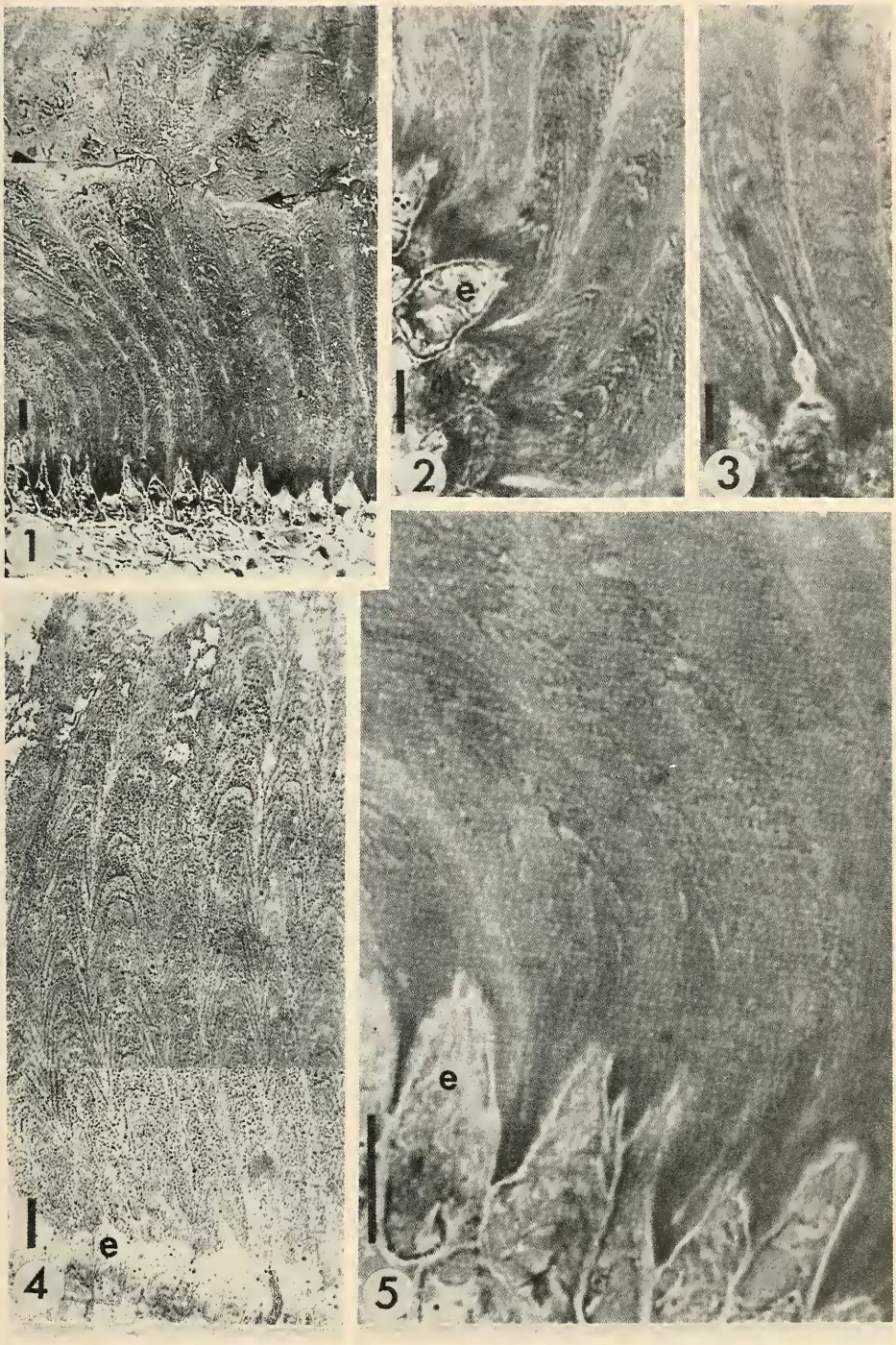


Fig. 13. E. collina; details of epidermal cells and cuticle lamellae. e, epidermal cell. In 1 arrows indicate abutment of cuticle of one primordium on that of another. Scale bars, all 100μm.

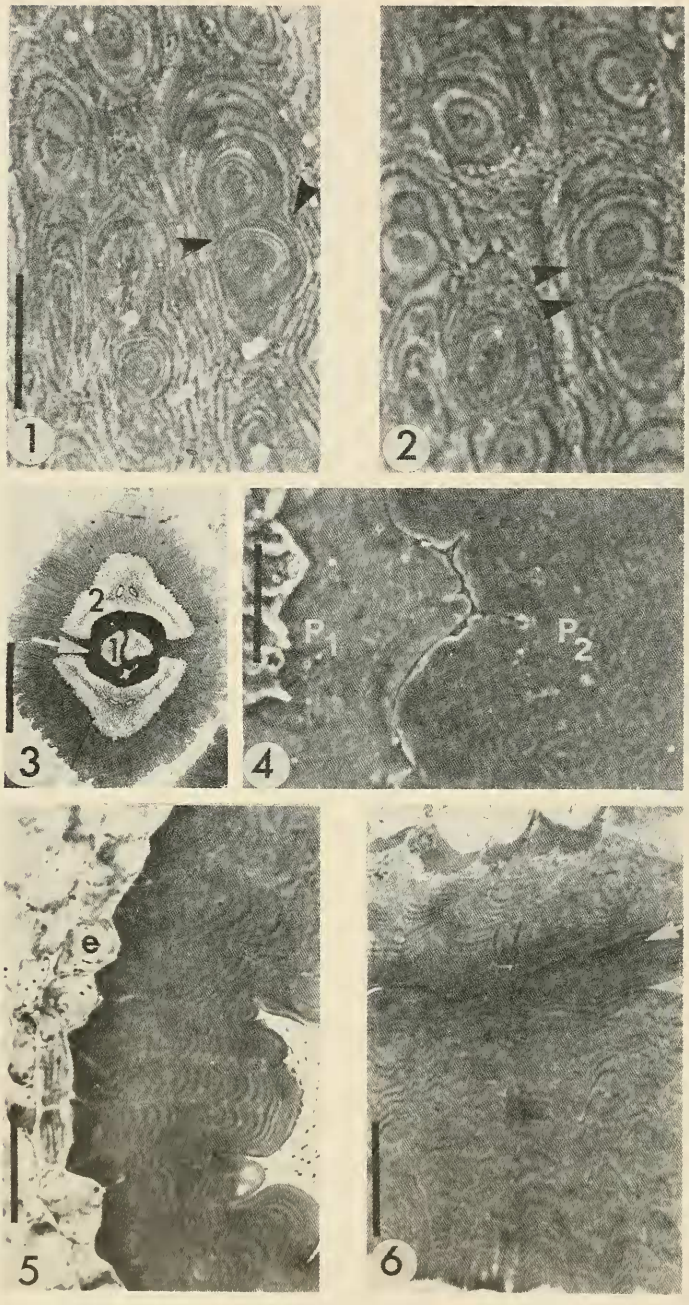


Fig. 14. E. collina, cuticle. **1** and **2**. Tangential sections of cuticle showing concentric sections of domes of the cuticle over individual cells. Arrowheads show the envelope of a single cell, now divided to give two cells. **3-6**. Transverse sections to show the adaxial cuticle (arrow in **1**) of leaf primordia (numbered as before). The layers of cuticle are much more compact and consequently less domed than those of the abaxial surface. These less wavy layers are maintained as the outer layers of the adaxial surface on an older primordium **6**. e, epidermal cell (its surface noticeably less ridged than those of the abaxial surface). Scale bars, *3* 1mm, all the others 100μm.

In our recently-published book, *Eucalyptus II*, we have given a full account of these methods of stomatal breakthrough in the *Corymbosae*. We have also dealt with a third and quite different method, which is that followed by stomata initiated on the outer surface of the flower buds, as well as on the outer (abaxial) surface of the petals.

Scurfiness of the cuticle

Among the other consequences of the presence of a layered, rubber cuticle in the *Corymbosae*, is the possibility of *scurfiness* of the cuticular surface. Glaucousness in eucalypts is due to coverings of epicuticular wax in the form of tubes on the surface of leaves or flower buds. No species of the *Corymbosae* have tubular wax; those which have epicuticular wax have it in the form of wax platelets. It follows that none of the species of the *Corymbosae* can be glaucous. However the flower buds and, in a few cases, the young leaves of some species have a silvery sheen, usually described as *scurfiness*. The origin of scurfiness is explicable in terms of the layered cuticles of the *Corymbosae*.

For its investigation, we have used a herbarium specimen of the tropical Western Australian species, *E. collina*. Fitzgerald, who discovered this species wrote of it that the branchlets and often the leaves appear 'as if covered with frost'. 'The trees can be seen at a great distance, owing to their silvery whiteness, which is a distinct character of the plant'. Dissections and transverse sections of leafy buds show that scurfiness is already apparent on the surface of the second smallest leaf primordia (P_2) and increases during the maturation of the leaves. After that, much of the scurfiness is gradually lost from the leaf surface, remaining, in the fully-developed leaves, mainly on the midrib and petiole. The innermost leaf primordia in the bud are tightly packed together forming what appears to be a solid mass, enclosed by the leaf cuticles (Fig. 12 [**1**]). For reasons we have already explained, and resulting from the close apposition of the adaxial surfaces of the innermost primordia, the abaxial cuticles become much thicker (at least initially) than the adaxial cuticles. The cuticle is strikingly layered, in much the same way as the cuticle of *E. maculata* (Fig. 13). On a P_4 leaf primordium, one can count 32 to 35 layers of the cuticle (Fig. 13 [**4**]). As in *E. maculata*, this suggests again a bilayer produced every day, assuming a plastochrone (interval between the production of successive leaf primordia) of about 8 days.

Each layer consists of a series of domes, each produced over a single epidermal cell (Fig. 13 [**1,2,5**]). Each of these domes joins laterally with those of adjacent cells to form an undulating layer (Fig. 14 [**4,5,6**]). Cut tangentially (Fig. 14 [**1,2**]), the cuticle presents the appearance of a series of concentric circles, the domes of individual cells cut at various levels. Where a cell has divided, these circles are enclosed in ellipses representing the now expanded and distorted domes of the original cell.

Scurfiness appears as outermost layers begin to break down and exfoliate (Fig. 15). The outer layers appear to become too brittle to stretch to accommodate the continued expansion to the surface of the leaf primordium. Possibly the rubber oxidizes prematurely and loses elasticity. Cracks appear in the domes (Fig. 15 [**2,3**]) and the cracks spread, allowing rafts of detached cuticle to form (Fig. 15 [**4**]). Spaces between the detached cuticular layers fill with air, the source of the silvery sheen of the scurfy cuticle.

Scurfiness of flower buds also arises from breakdown of the outer layers of rubber cuticle, as is discussed and illustrated in *Eucalyptus II*. Deep fissures develop in the cuticle and rafts of the outer layers may exfoliate.

Discussion

Following its initiation, the GMC ceases to form rubber. Indeed, its outer surface may be coated with a laticase. We had already shown for other eucalypts (not *Corymbosae*)

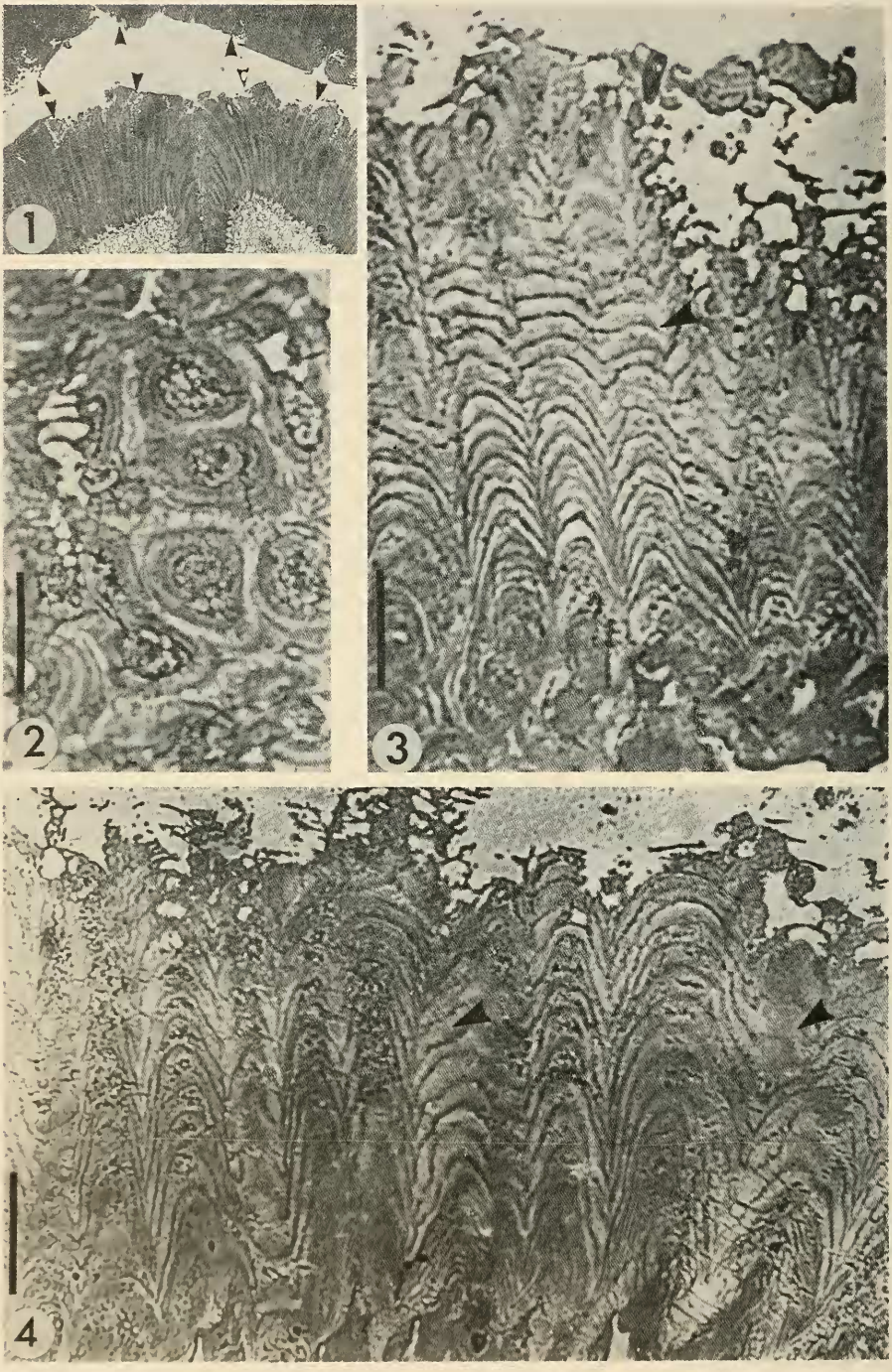


Fig. 15. E. collina. Development of scurfiness of the cuticle. **1.** Location of early scurfiness (arrowheads). **2.** Tangential section showing the erosion and breakup of the columns of the outermost cuticle lamellae. **3** and **4.** Swelling of the light zones (arrowheads) and breakaway of the outer layers. Scale bars, **1** 1mm, all the others 100μm.

that, following its initiation, the GMC ceases to make cuticle above itself (Carr and Carr, 1978). Next, the guard cell initials which result from the division of the GMC begin to produce cuticularized layers within the mother cell envelope. The sequence of production first, of rubber layers, then of cuticularized layers of the cuticle is also that followed by the ordinary cells of the epidermis. This dual potentiality may have been a feature initially common to the ancestors of all the eucalypts but the capacity to produce rubber was reduced or lost in most groups of eucalypts. In some species perhaps only a few of the outermost layers of the cuticle consist of rubber (giving the cuticle a characteristic sheen) while the bulk of the cuticle consists of cuticularized layers. A rather similar situation is found developmentally in the leaves of certain deciduous species of the *Corymbosae* in the tropics. Following a few weeks of leaflessness, a new suite of thin, pale green leaves is produced which expand rapidly over a few days then gradually become thicker and darker green. In such leaves the initial cuticle of rubber is relatively thin and paucilamellate and, following the attainment of full size, a relatively thick cuticularized cuticle is developed to reinforce the protective layers. On this hypothesis, the rubber cuticle of the *Corymbosae* represents the survival of an ancient device for the protection of the leaf during its early expansion. This has given way, in most species of eucalypts, to the production of the less-overtly layered, cuticularized cuticles which are also a feature of most angiosperms.

The flower is considered by morphologists to be a 'conservative' organ in relation to evolution. In the flowers of the *Corymbosae* only a rubber cuticle, which is in many instances remarkably thick and is always layered like that of the leaves, is formed. This supports the hypothesis that the rubber cuticle is, in an evolutionary sense, an older covering than the cuticularized layers. One can visualize that a rubber cuticle would present advantages to tropical species which, either deciduous or semi-deciduous, must rapidly expand a suite of leaves while still retaining a protective cuticle over their surfaces. Unfortunately we know little about the cuticles of tropical plants, but it seems not unlikely that rubber cuticles may be found to be present in genera other than *Eucalyptus* and *Angophora*.

A host of questions is raised by these findings. Are the bilayers really diurnally produced, and if so which of the two constituent layers is produced during the day and which during the night? Is layering suppressed in continuous light, as it is in insect cuticles and the cell walls of cotton hairs? Why are the cuticles of some species and some organs scurfy, those of other species and other organs not? Is the eventual hardening of the initially elastic cuticle due to oxidation, as was suggested by Smith (1908)? Perhaps scurfy cuticles lack antioxidants, such as α-tocopherol, a known constituent of eucalyptus oil; alternatively, since scurfy cuticles lack epicuticular wax, perhaps it is this lack which permits the development of scurfiness.

Qualitative and quantitative aspects of the phytoglyph in *Corymbosae*

In 1883 Ferdinand von Mueller wrote, concerning the plant fossils found in association with the deep leads of the Victorian goldfields, that: 'By the aid of the microscope we may yet hope to be able to obtain characteristics of diagnostic value from the anatomy of leaves sufficiently positive to recognize ordinal and even perhaps generic groups'. 'How far this can be done even with living plants remains yet to be studied: but I was enabled, for instance, to demonstrate the occurrence of Epacridae in New Guinea from the microscopic comparison of the leaf epidermis, brought from thence without flowers or fruits, with the very peculiar cuticle of many Epacridae easily recognized microscopically'. In his *Eucalyptographia* Mueller illustrated features of the leaf epidermis of 39 species of eucalypts. He classified them as *isogenous*, with roughly equal numbers of

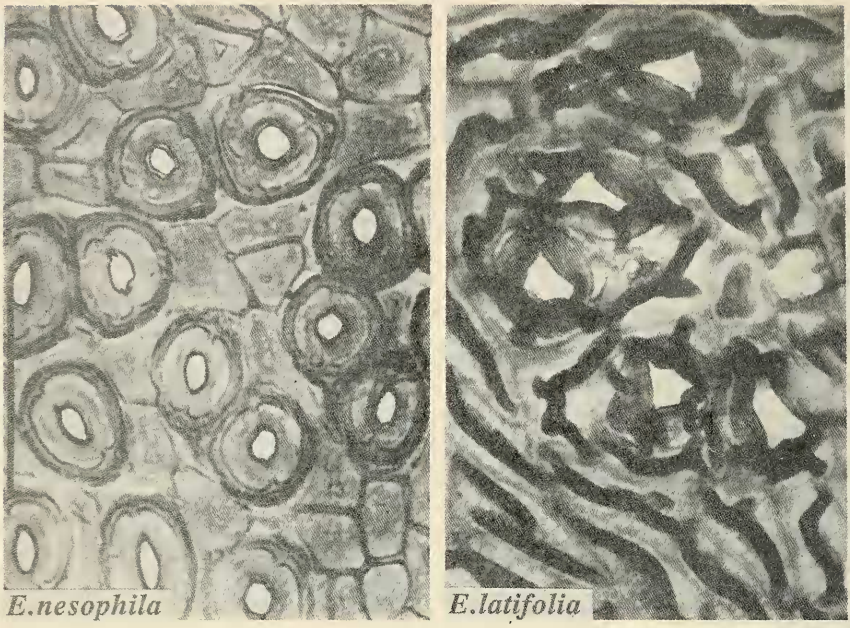


Fig. 16. E. nesophila and *E. latifolia*, showing different patterns of cuticular ornamentation. (Figs 16-23 all light micrographs of stained cuticles; in comparisons always the same surface, upper or lower of the two species, at the same magnification is shown.) Scale bar: 50μm.

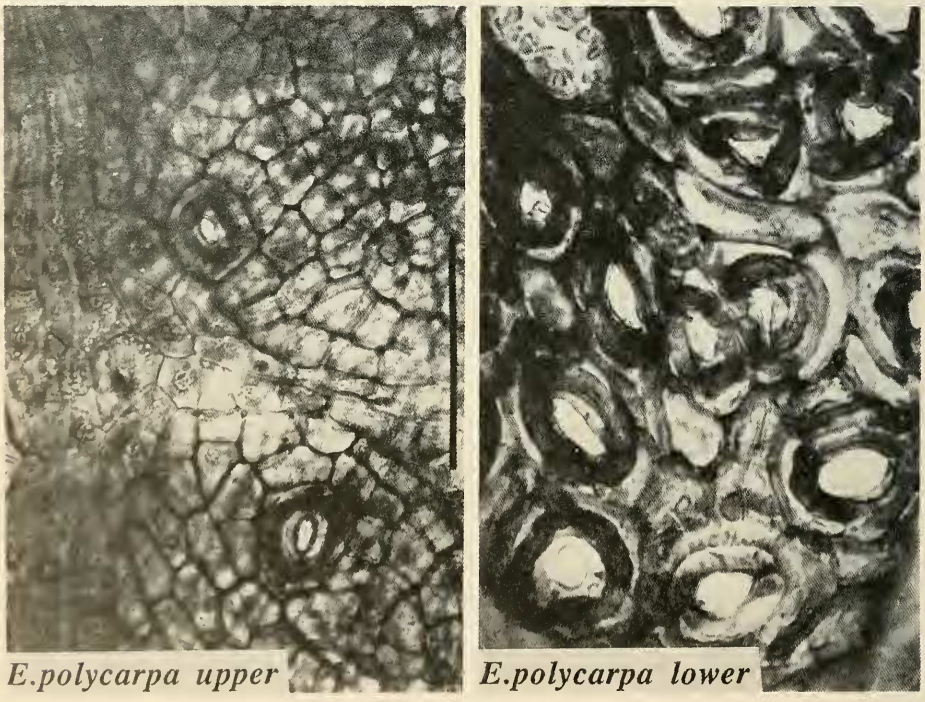


Fig. 17. E. polycarpa, showing heterogenous arrangement of the stomata. Scale bar: 50μm. See also legend to Fig. 16.

stomata on both upper and lower surfaces, *heterogenous*, with many fewer on the upper than on the lower, or *hypogenous*, with stomata only on the lower surface.

This excellent beginning of the study of the microscopic features of the leaves of Australian plants suffered a mortal blow when Maiden (1909-1928), after quoting Mueller's stomatal classification, dismissed the study of stomata with the remark (our emphasis) that '*the method cannot be used for diagnostic purposes because of the variation in the distribution of stomata even in the same tree*'. Maiden offered no evidence of such variation and indeed made no further reference to stomata in his voluminous writings. Since that time no-one paid any attention to the features of the epidermis of eucalypt leaves until, in 1971, we published an account of our studies (Carr *et al.*, 1971) of the leaves of the various 'type materials' supposed by Blake (1953) to be all of a single species, *Eucalyptus dichromophloia*. We were able to demonstrate that these materials belonged to no fewer than five different species. Blake had based his description of the juvenile foliage of *E. dichromophloia* on leaves of a species of *Angophora*, included in the folders of one of these species of eucalypts. Recently, in our new book, *Eucalyptus II*, we have shown that the type folders of *E. erythrophloia* (which included the *Angophora* leaves) also contain a specimen of leaves of yet another species (*E. ellipsoidea*), which is described for the first time. Thus the type folders of *E. erythrophloia* contain specimens, all collected in the same locality (where they still occur), of no fewer than three species belonging to two genera! The methods we used included scanning electron microscopy of the leaf cuticles, and light microscopy of stained cuticle preparations and of thin sections of cuticles. These approaches make it possible to reconstruct a 3-dimensional view of the cuticular patterns of the leaves and an analysis of the positions, shapes and types of cells in the leaf epidermis. For the totality of the information thus obtained we coined the term '*phytoglyph*', which means literally 'plant fingerprint'. We maintain that, just as human fingerprints may be used diagnostically to identify persons, so the phytoglyph may be used to identify species of eucalypts, at least in some groups.

Following the work on '*E. dichromophloia*', we used the method to identify old specimens, collected by Leonard Brass in Cape York Peninsula (and which had been filed under *E. dichromophloia*) as *E. nesophila* (S.G.M. Carr, 1972). At that time, this species was thought to be endemic to the Northern Territory. Dr Nigel Wace then presented us with a problem in the form of a small leafy shoot collected on Fenelon Island, off the coast of South Australia. We identified the material as belonging either to *E. socialis* or to a closely related, possibly undescribed, species (Carr and Carr, 1976). At about the same time, that species, *E. yalatensis*, was described by C. D. Boomsma. Our further studies showed that the species of the informal group, the *Bisectae*, have several peculiarities of the leaf cuticle. In some species, during stomatal breakthrough, the cuticle becomes eroded into sinuous canyons or crypts, at the bottom of which lie groups of stomata (Carr and Carr, 1978, 1980b, 1980c). Other species of the *Bisectae* have special bars of cuticle forming the line of closure of the stomata (Carr and Carr, 1979). Yet others have peculiar ingrowths of cuticle in the anterior chambers of the stomata (Carr and Carr, 1980a). These features of the *Bisectae* were shown to be consistent with taxonomic relationships within that group. While such studies showed the usefulness of phytoglyphic methods, but by far the most successful and extensive use we have made of those methods has been in clearing up what M. R. Jacobs termed 'the bloodwood puzzle', i.e. the problem posed by the recognition and identification of the confusing and numerous tropical species of the *Corymbosae*. We have published accounts of these species in our two books, *Eucalyptus I* and *Eucalyptus II*. In doing so we refrained from adding to the volume of descriptive data by including all the data from the immensely detailed

phytoglyphic studies which largely enabled us to sort the hundreds of available specimens into groups which formed the basis of species descriptions.

In what follows we now provide a resume of the sorts of information which can be obtained from phytoglyphic studies of the *Corymbosae* and which can be used as aids to species identification. All the photographic illustrations are light micrographs of stained leaf cuticles.

Qualitative features of the leaf epidermis in *Corymbosae*

A wide variety of phytoglyphic features is shown by different species of the *Corymbosae*. The cuticular ornamentation of the subsidiary cells of the stomata may be very simple, as it is in the Eximiae (or yellow bloodwoods) and in, e.g. *E. nesophila*, or it may be very complex, as in e.g. *E. latifolia* and *E. capricornia* (Fig. 16). The ordinary, non-stomatal cells may contribute to the pattern of ornamentation (as they do in *E. latifolia*) or have little or no ornamentation. The illustration of *E. latifolia* was made from a specimen of that species from Papua-New Guinea, and the pattern matches exactly that of specimens from Australia. Similarly the illustration of *E. nesophila* is from a specimen from Cape York Peninsula and the ornamentation is exactly the same as that of specimens from the Northern Territory and Western Australia. The pattern is a constant feature of the species and within limits, does not vary over the entire range of the species or in cultivation. Moreover, it is constant over time, so that specimens collected during the last two centuries may be compared and identified with recently-collected specimens.

As Mueller was aware, some eucalypts have no stomata on the upper surface of the leaf, or may have many fewer stomata on the upper surface than on the lower. Contrary to what Maiden wrote, these are constant characteristics for the species. For instance, *E. polycarpa* has scattered stomata on the upper surface of the leaf with a stomatal density of about 1 per square millimetre (Fig. 17), except near the margins and midrib where the density is about 3 per square millimetre. (Other, related species, have either no stomata on the upper surface or higher densities of stomata than *E. polycarpa*).

The cap cells of oil glands have rather thin, unornamented cuticles. In the *Corymbosae* they are arranged in characteristic groups of 3 or more per oil gland and so are easily recognized in the light microscope. Some species, such as *E. terminalis* have large numbers of groups of cap cells on both sides of the leaf; others (e.g. *E. ollaris*, *E. opaca*) have few or none (Fig. 18). There may be differences between upper and lower surfaces. For instance *E. polycarpa* has very few such groups on the upper surface, but large numbers on the lower surface. Similarly the actinocytic 'giant stomata' may be less common on the upper surface than on the lower; some species (e.g. *E. opaca*, *E. centralis*) have relatively few giant stomata on either surface of the leaf, while other species (e.g. *E. terminalis*) have many on both surfaces. We shall make further reference later on to 'giant stomata'. Finally, the stomata of some species are in general larger than those of other species (Fig. 19).

If studies of macroscopic morphology such as fruit shape and size, features of the flower, leaf shape and arrangement etc. suggest that two specimens are of the same species, we may expect that their phytoglyphic features will be identical. In *Eucalyptus II* we have shown that the specimens which (wrongly) have been called *E. perfoliata* have features of the flowers which are identical with those of *E. lamprocalyx*. The fact that the phytoglyphic features of these specimens are identical with those of *E. lamprocalyx* (Fig. 20) supports the hypothesis of identity, and since the only validly-published name is that of *E. lamprocalyx*, it is under that name that we must now file specimens previously termed '*E. perfoliata*'.

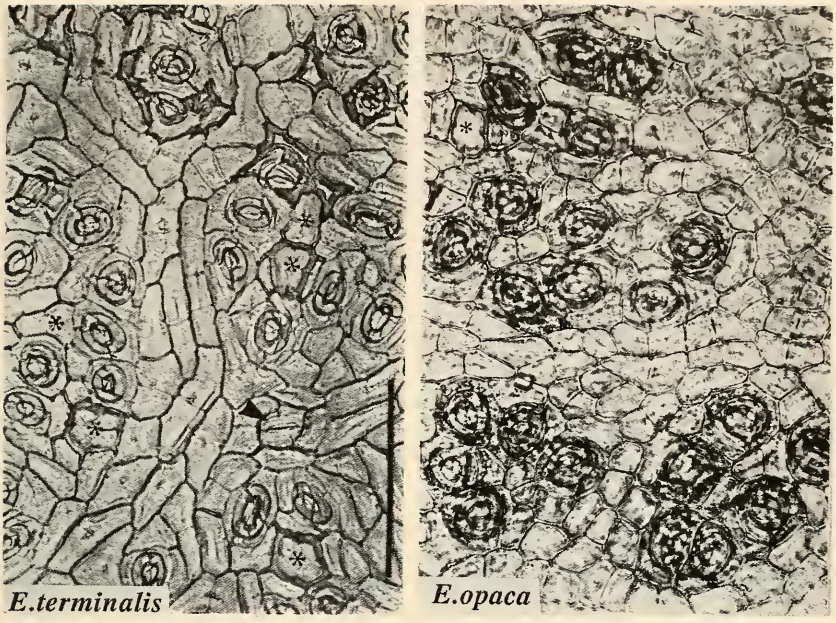


Fig. 18. E. terminalis, with many groups of oil gland cap cells (asterisks) and *E. opaca* with only one. Arrowhead, giant stoma of *E. terminalis*. Scale bar: 500μm. See also legend to Fig. 16.

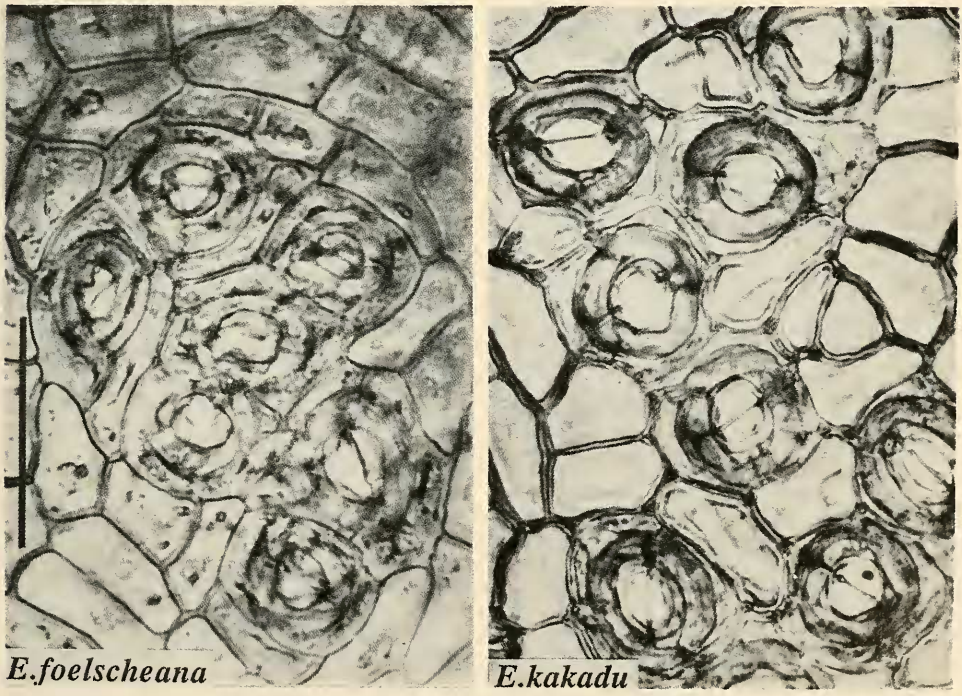


Fig. 19. E. foelscheana and *E. kakadu*, to show differences in size of stomata. Scale bar: 50μm. See also legend to Fig. 16.

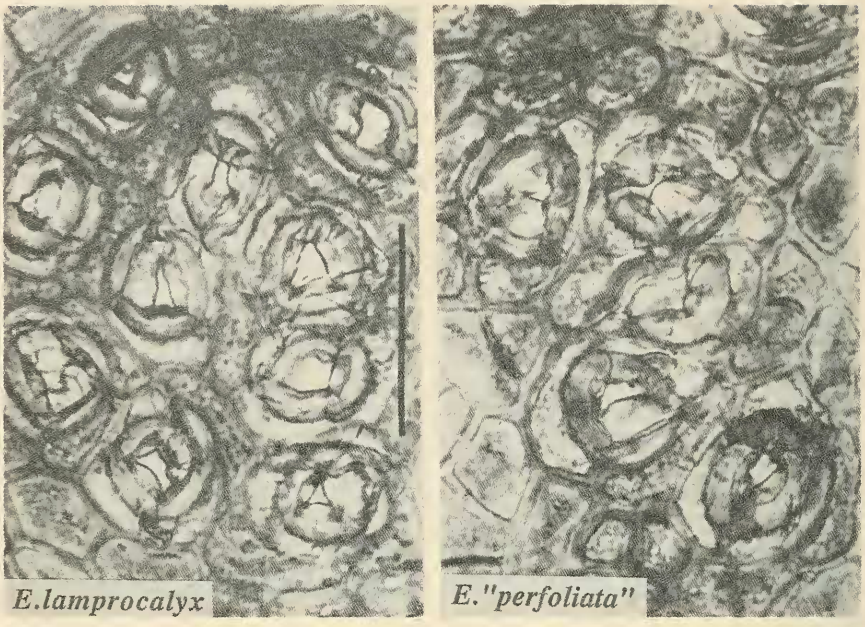


Fig. 20. E. lamprocalyx (isotype) and *E. 'perfoliata'*. Identical phytoglyphs supporting identity of specimens. Scale bar: 50μm. See also legend to Fig. 16.

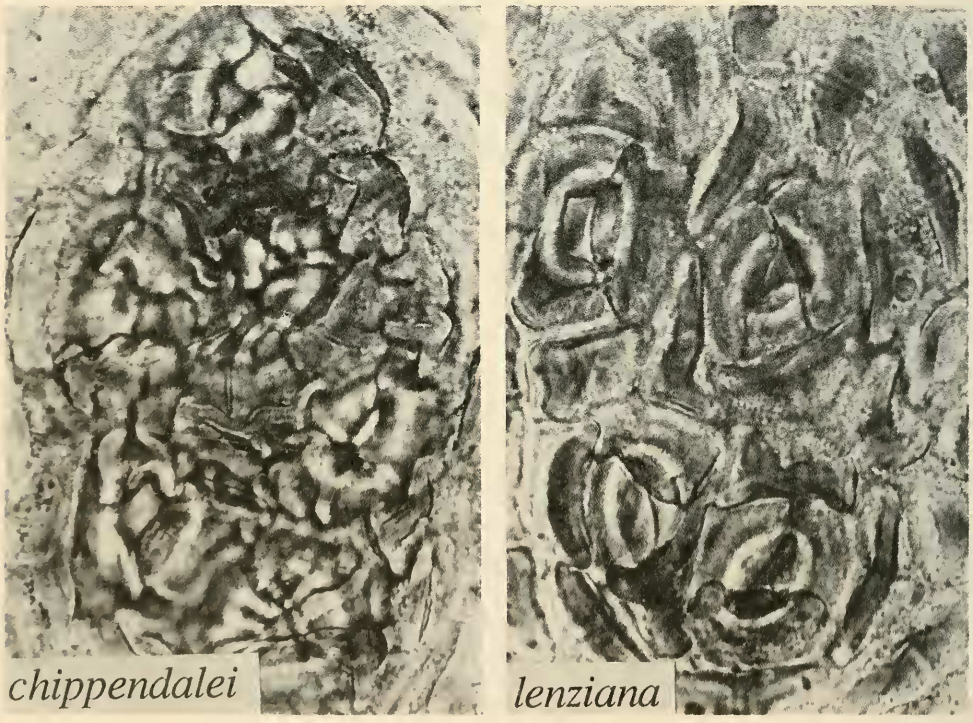


Fig. 21. E. chippendalei, with well-ornamented cuticle and *E. lenziana* with poorly ornate cuticle. Scale bar: 50μm. See also legend to Fig. 16.

On the other hand, the cuticular ornamentation of closely related species, such as *E. lenziana* and *E. chippendalei*, may be quite different in appearance (Fig. 21). These differences may therefore be of very great diagnostic value, especially when the two species (the relationship between which may be evident, as they are in this case, from similarities of a number of macroscopic features) occur together in the same region.

These examples are intended to show how phytoglyphic features, taken together with comparisons of the macroscopic features of leaf, fruit and flower can be used to establish identity between specimens of the same species or, alternatively to establish that they are of different species. Since Mueller's time, many publications have appeared testifying to the usefulness of such microscopic features of the leaves in taxonomy. Since palaeobotanists often have only fossilized leaves to work with, they have pursued the study of such features much more intensively than taxonomists of living plants. Unfortunately, all too few Australian botanists, other than one or two palaeobotanists, have paid any attention to these features of either living plants or fossil leaves. For both Monocotyledons and Dicotyledons it is now, however, *the general consensus that the pattern and shape of the leaf epidermal cells and features of their cuticles are species-specific and differ from one taxon to another* (Barthlott and Ehler, 1977).

Cell patterns involve the distribution of different types of epidermal cells and their positional relationships to each other and to the underlying tissues (e.g. of the veins) (Barlow and Carr, 1984). The presence and the distribution of idioblasts other than stomata (e.g. the cap cells of oil glands, emergent oil glands, hairs etc.) in the epidermis affects these patterns in species-specific ways. For instance, the first-formed stomata of the leaf are so-called 'giant stomata'. They arise near the margin and near the midrib, positions on the leaf primordia which remain fixed, relatively to the rest of the lamina, since marginal meristem activity in eucalypt leaf primordia ceases very early in development. Later on, some more giant stomata may be laid down in the intervening and expanding region of the lamina. In this region, the giant stomata may occupy positions near the centre of vein islets or over the junctions of smaller veins. These are also sites at which epidermal cells may differentiate to form the initials of oil glands. Oil gland initials may also arise early in leaf development near the margin and near the midrib. Thus in both situations, there is some interchangeability between the initials of oil glands and giant stomata, idioblasts which therefore compete, as it were, for preferred positions in the epidermis. On the other hand, neither oil glands nor giant stomata are a constant feature of the pattern and some species (e.g. *E. opaca*) exhibit a dearth of both in their leaf cuticles.

We have already referred to 'giant stomata' as actinocytic, i.e. they generally have a halo of numerous subsidiary cells so that they differ in appearance from the ordinary stomata. Such giant stomata were reported to be a feature of a number of Myrtaceae by Bandulska (1923; 1928-31), who found them on fossilized leaves of Myrtaceae in the Eocene of the London Clay deposits. Solereder (1908) who also reported the existence of giant stomata in some mangrove species referred to them as 'water stomata'. This is a misnomer. There is no evidence as far as we are aware, of a function of these stomata in secreting water. Nevertheless, some recent writers, including Stace (1965) have persisted in using the term 'water stomata'. Van Cotthem (1971) refers to them as hydathodes (in e.g. *Buxus* spp.). Van Wyck *et al.* (1982) also refer to 'water stomata' of the leaves of South African species of *Eugenia*. Napp-Zinn (1973-74) doubts the existence of giant stomata and declares his astonishment that Sitholey and Panda (1971) report on the giant stomata of *Mangifera indica* and *Limonia acidissima*, without giving measurements of their actual size. Indeed, in terms of the length of the guard cells, the so-called giant stomata may, in some cases, not be larger than the ordinary stomata. Solereder reported 'water stomata' as either larger than or smaller than, the ordinary stomata. Nevertheless,

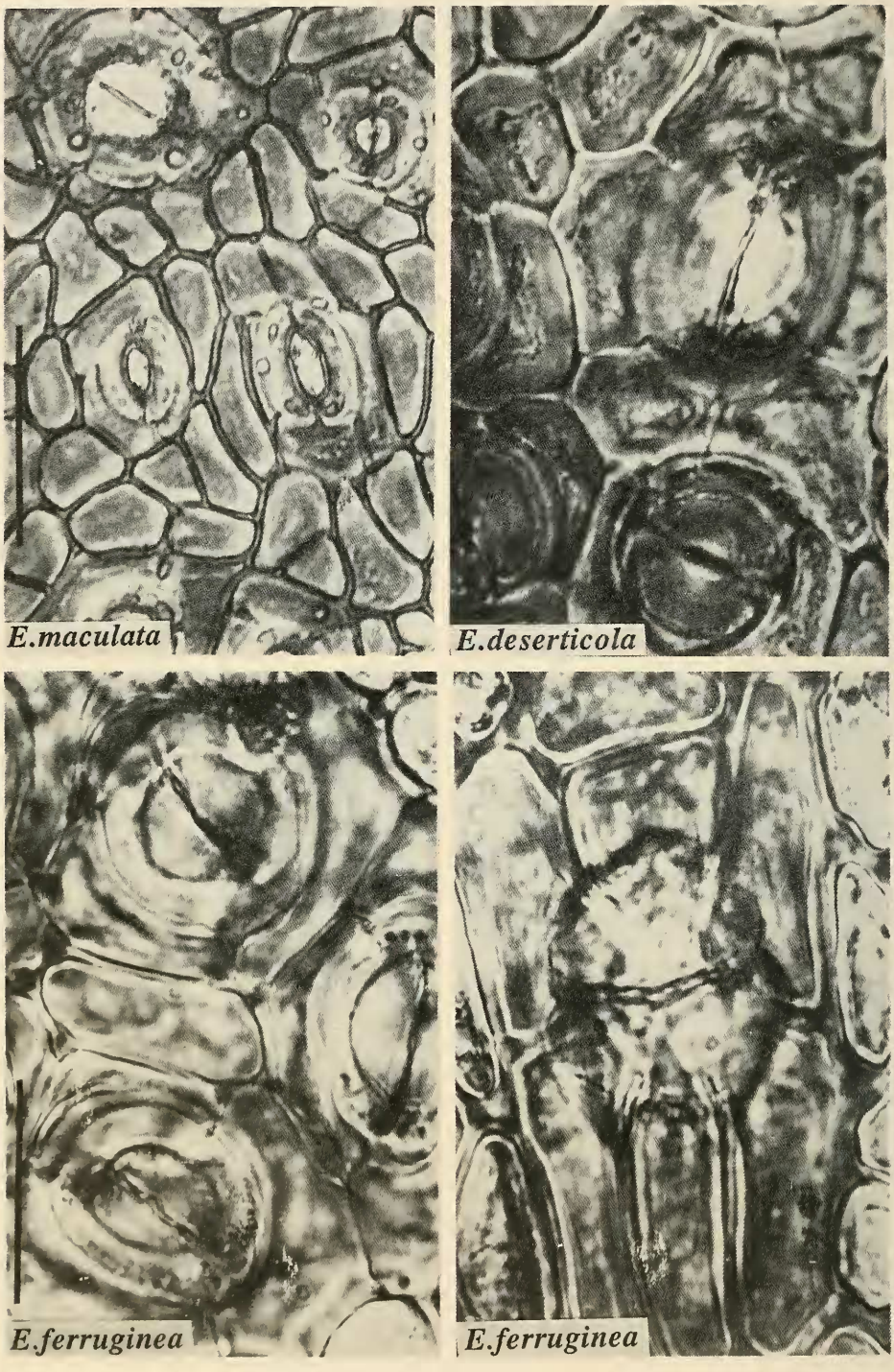


Fig. 22. Giant stomata of *E. maculata*, *E. deserticola* and *E. ferruginea*, in comparison with ordinary stomata of these species. Scale bar: 50μm. See also legend to Fig. 16.

because of their larger complement of subsidiary cells, the giant stomata do stand out as different from — and their guard cells are indeed in most cases larger than — the ordinary stomata (Fig. 22). This is the case in many of the species of the *Corymbosae*. In addition, the cuticular ornamentation of the subsidiary cells of giant stomata is often less ornate or well-developed than that of the subsidiary cells of ordinary stomata.

Quantitative aspects of phytoglyphic features of the *Corymbosae*

We have already touched on two quantitative aspects of the phytoglyphic features of the leaves, *viz*, differences in stomatal size between two species and the possibility of a very low stomatal density of the upper surface of the leaves in some species. Between related 'heterogenous' species (to use Mueller's term) the density (number per unit area) of the stomata on the upper surface may be quite different. *E. derbyensis*, for instance has up to 90 stomata per square mm, as compared to the 1 per square mm in the related *E. polycarpa*.

Frequency-spectra of the subsidiary cells

The difference between giant and ordinary stomata in the number of subsidiary cells is not the only such difference. Even among the ordinary stomata some will have 3, some 4, some 5, etc. subsidiary cells. If one makes counts of the subsidiary cell complement of a hundred stomata the percentage of 3s, 4s or 5s, etc. appears to be a reproducible characteristic of the species. For instance even a glance at a cuticle of a specimen of *E. centralis* shows that most of its stomata have 3 subsidiary cells (Fig. 23), whereas in a

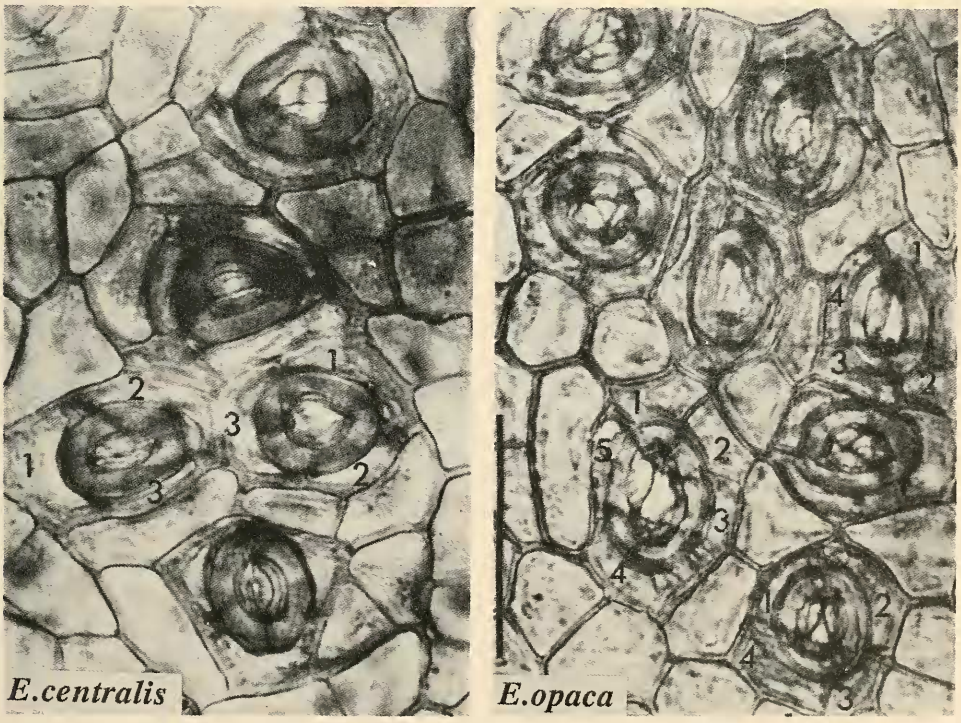


Fig. 23. E. centralis. All stomata in field each with 3 subsidiary cells, to compare with *E. opaca*, some stomata with 4, some with 5 subsidiary cells. Scale bar: 50μm. See also legend to Fig. 16.

similar cuticle preparation of the related *E. opaca*, there are more usually 4 or 5 subsidiary cells to each stoma. Before we consider the exciting possibilities of the diagnostic uses of such *frequency-spectra* of subsidiary cells we must first enquire into their constancy or otherwise in a single leaf, in different leaves of a single specimen, and between different specimens of a single species. Taking the latter first, we see (Table 1) that between 5

TABLE 1

Eucalyptus centralis
5 different specimens

	lower				upper			
Subsidiary cells (%)	3	4	5	6	3	4	5	6
Specimen								
Ford 50	90	7	1	0	83	17	0	0
Carr 752 (type)	89	11	0	0	84.5	15	0.5	0
Jacobs 143	90	11	0	0	80	16	4	0
George 12954	84	15	1	0	84	15	1	0
Frith 49	86	12	2	0	79	18	3	0
Means	87.8	11.2	0.8	0	82.1	16.4	1.7	0
χ^2	0.066	2.929	—		0.312	0.427	—	
	$P > 0.95 < 0.98$		n.s. ($P < 0.90$)		$P > 0.99$		$P = 0.98$	

specimens of *E. centralis* there are no differences between percentage of 3s to a very high level of probability. The very much smaller percentages of 4s and 5s would require very large samples to be taken (perhaps 500 or 1000) to show whether or not the percentages are repeatable from specimen to specimen. The figures shown are based on counts numbering between 100 and 200. Another feature is shown by the table: the upper surface generally has a lower percentage of 3s than the lower. The percentages of both 3s and 4s on the upper surface is the same in all the specimens, to a very high degree of probability. The frequency-spectra of 6 specimens of *E. centralis* are shown in the graphs (Fig. 24). Evidently the spectra are highly reproducible from one specimen of a given species to another.

The method of sampling to obtain the data was, using an oil-immersion objective and a stained, *inverted* cuticle preparation, to count the subsidiary cells of two or three stomata in a microscope field, then move to another field, repeating the process until over 100 counts had been obtained. By subsidiary cells, we mean all the cells which have a wall or part of a wall in common with one or both of the guard cells (see below, for a discussion of this definition). If possible, the preparations for this purpose were taken from the middle third of the leaf. Will any leaf from a given specimen suffice for this procedure? To test this, preparations were make from 5 leaves of a single specimen and counted. The data (Table 2) show that the 5 leaves yield frequency-spectra which are, to a very high degree of probability, identical. Again, the same sorts of differences between upper and lower surfaces are apparent, as in Table 1. There is a tendency for the spectra of the upper surface to be shifted along the abscissa, as compared with the lower. But this is not universally the case, as experience has shown us.

If the middle portion of a leaf is not available, will it make a difference if we use some other part? To test this a single leaf was cut into 5 transverse strips, tip to base. Evidently (Table 3) there is no statistical difference, to a high degree of probability, between the tip, the middle and the base. But a word of caution is necessary. Both tip and base are narrower than the middle and in them the margin and midrib come closer

TABLE 2

Eucalyptus orientalis Carr 763 (type)
5 different leaves

	lower				upper			
Subsidiary cells (%)	3	4	5	6	3	4	5	6
Leaf 1	60.4	31.6	6.4	1.6	47.5	44.17	6.6	1.6
Leaf 2	57.89	32.46	7.29	1.7	49.19	42.74	8.1	0
Leaf 3	58.6	32.3	6.77	2.25	48.62	40.37	10.1	0.9
Leaf 4	57.6	35.2	6.4	0.8	48.8	47.4	3.15	0.78
Leaf 5	58.18	36.36	6.4	0	49.8	44.53	4.45	1.2
Means	58.53	33.178	7.74	—	48.78	43.84	6.47	—
χ^2	0.0865	0.49127	1.5247		0.0588	0.065	4.75559	
	$P>0.99$	$P>0.95$	$P>0.90<0.95$		$P>0.99$	$P>0.95<0.98$		n.s

TABLE 3

Eucalyptus ollaris
5 zones, tip to base, of a single leaf of Carr 827

	lower				upper			
Subsidiary cells (%)	3	4	5	6	3	4	5	6
Zone 1 (tip)	72	25.4	2.6		62.7	28.4	8.8	0
Zone 2	72.75	24.62	2.625	0	62.2	35.43	2.36	
Zone 3	73	24	3	0	64.6	27.4	7.96	0
Zone 4	70.37	26.85	2.77	0	63.41	31.45	3.23	1.6
Zone 5 (base)	72.41	25.82	1.76	0	61.19	30.59	8.2	0
Means	72.17	25.338	3.88	—	62.88	30.65	6.11	—
χ^2	0.01825	0.19054	—		0.11129	1.9305	—	
	$P>0.9$	$P>0.99$			$P>0.99$	n.s. ($P<0.90$)		

together. These latter are regions in which there are concentrations of giant stomata, which, with their unusually large number of subsidiary cells, could affect the comparisons we wish to make. In addition, there might be 'edge-effects' on the frequencies of subsidiary cells even in ordinary stomata in the vicinity of the margin and midrib. These effects could be quite disturbing in especially narrow leaves (e.g. those of *E. nelsonii* and *E. fordeana*). To test for these edge effects we made use of leaves of *E. opaca*, which as we have already mentioned, have relatively few giant stomata. The stomata were sampled close to the margin and close to the midrib and the data compared with counts made half-way between those regions (Table 4). Evidently there is an appreciable edge-effect, especially on the upper surface, where the percentage of 3s is only two-thirds that of the stomata of the intervening region. The recommendation is, therefore, to avoid counting close to the margin and midrib.

Use of the frequency-spectra in the *Corymbosae*

We have made great use of this quantitative aspect of the phytoglyph. It is particularly useful in distinguishing between specimens of two species from the same general area. For instance, *E. centralis* (with a preponderance of 3s) is readily distinguishable (Fig. 24) from *E. opaca* (Fig. 25), which occurs together with it in the same regions of central Australia and the Great Sandy Desert. The specimens used for these graphs

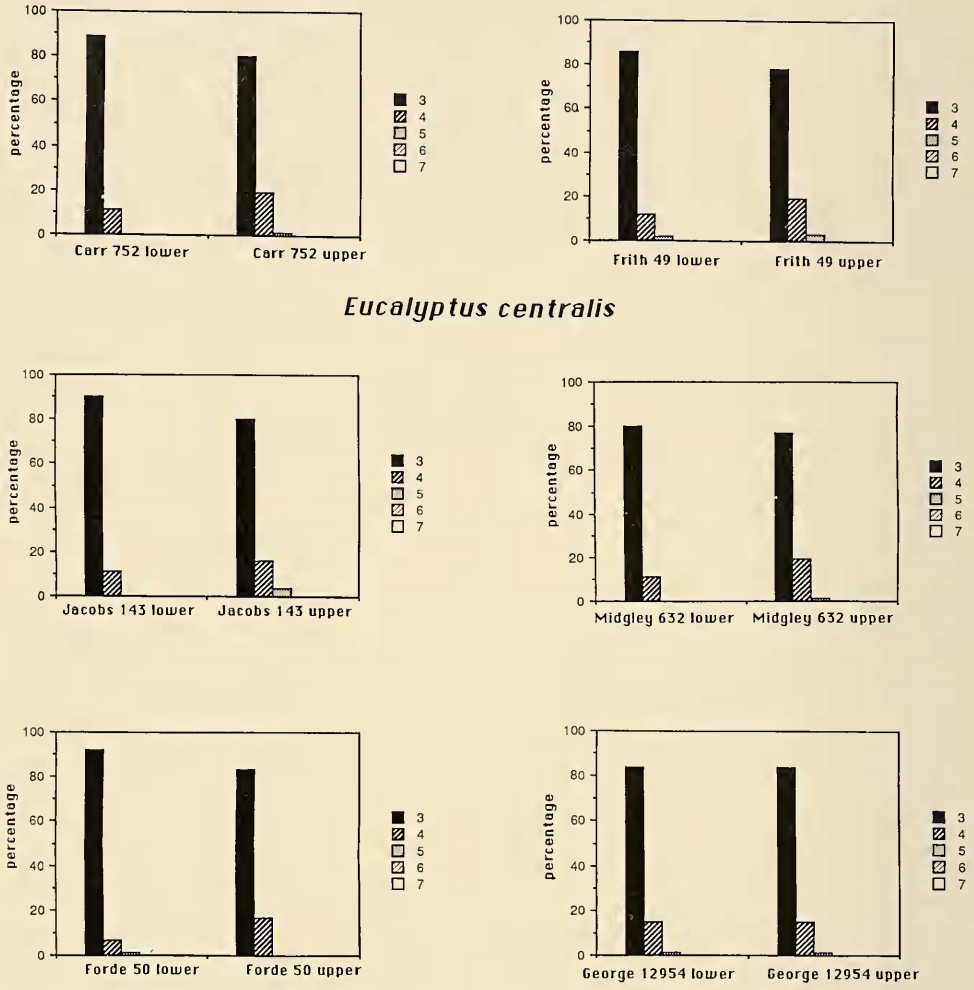
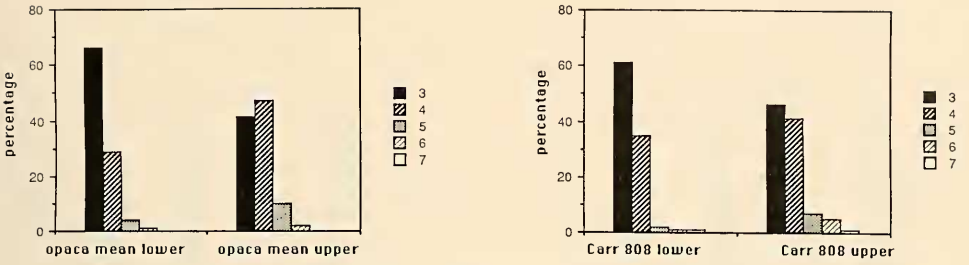
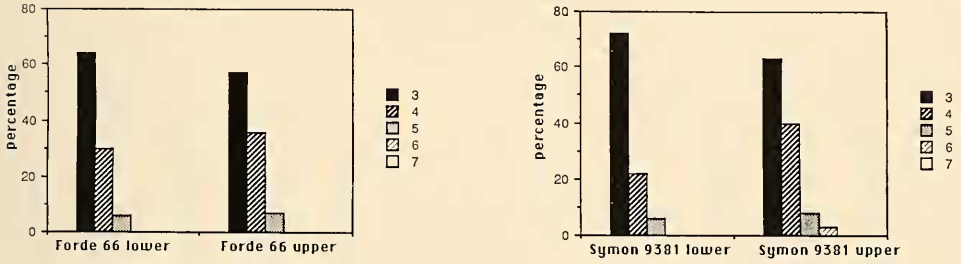


Fig. 24 and *Fig. 25* (opposite). Frequency spectra of *E. centralis* and *E. opaca*. Each separate graph represents a single specimen or the means of several specimens. On the left of each graph, the frequency-spectrum (f-s) of the lower surface, on the right the f-s of the upper surface.

were drawn from widely separated regions, in the Northern Territory and in Western Australia. Specimens of three other central Australian species, *E. eremaea*, and the mountain top mallees, *E. nelsonii* and *E. fordeana*, are also readily distinguishable by their frequency-spectra (Fig. 26). The Forrest specimen of *E. fordeana* was collected in 1883 by the explorer, John Forrest, on the summit of Mt Augusta in Western Australia, a locality very distant from the localities in central Australia from which the other specimen was obtained. Nevertheless, the frequency spectra match. The frequency spectra of widely disjunct specimens of three other central Australian species, *E. symonii*, *E. australis* and *E. connerensis* (Fig. 27) are also consistent and enable the specimens to be grouped unequivocally.



Eucalyptus opaca



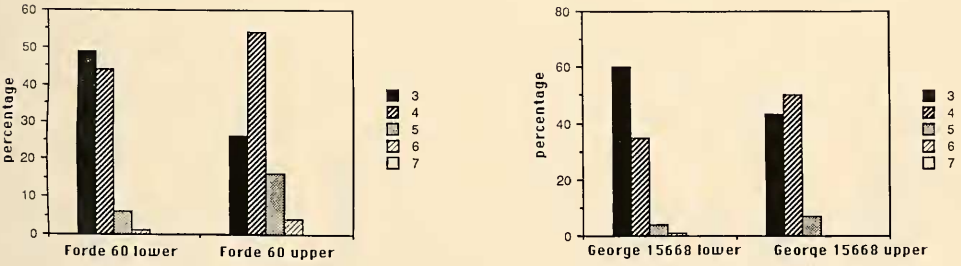


TABLE 4

Eucalyptus opaca Carr 808

Edge effects: counts near the midrib, near the margin and in intermediate regions of the leaf lamina

	lower					upper			
Subsidiary cells (%)	3	4	5	6	7	3	4	5	6
intermediate	68.57	27.6	2.86	0.95	0	62.39	34.19	3.4	0
near midrib	62.7	34.9	1.58	0.79	0	39.27	48.2	9.82	2.68
near margin	57.27	36.36	4.545	0.9	0.9	38.68	54.72	6.6	0
Means	62.85	32.95	2.995	0.9	–	46.78	45.70	6.6	–
χ^2	0.038	0.8687	0.006	–		5.2089	2.8989	–	
	$P>0.95$	<0.98	n.s. $P<0.90$	$P>0.99$		n.s. ($P<0.05$)		n.s. ($P<0.05$)	

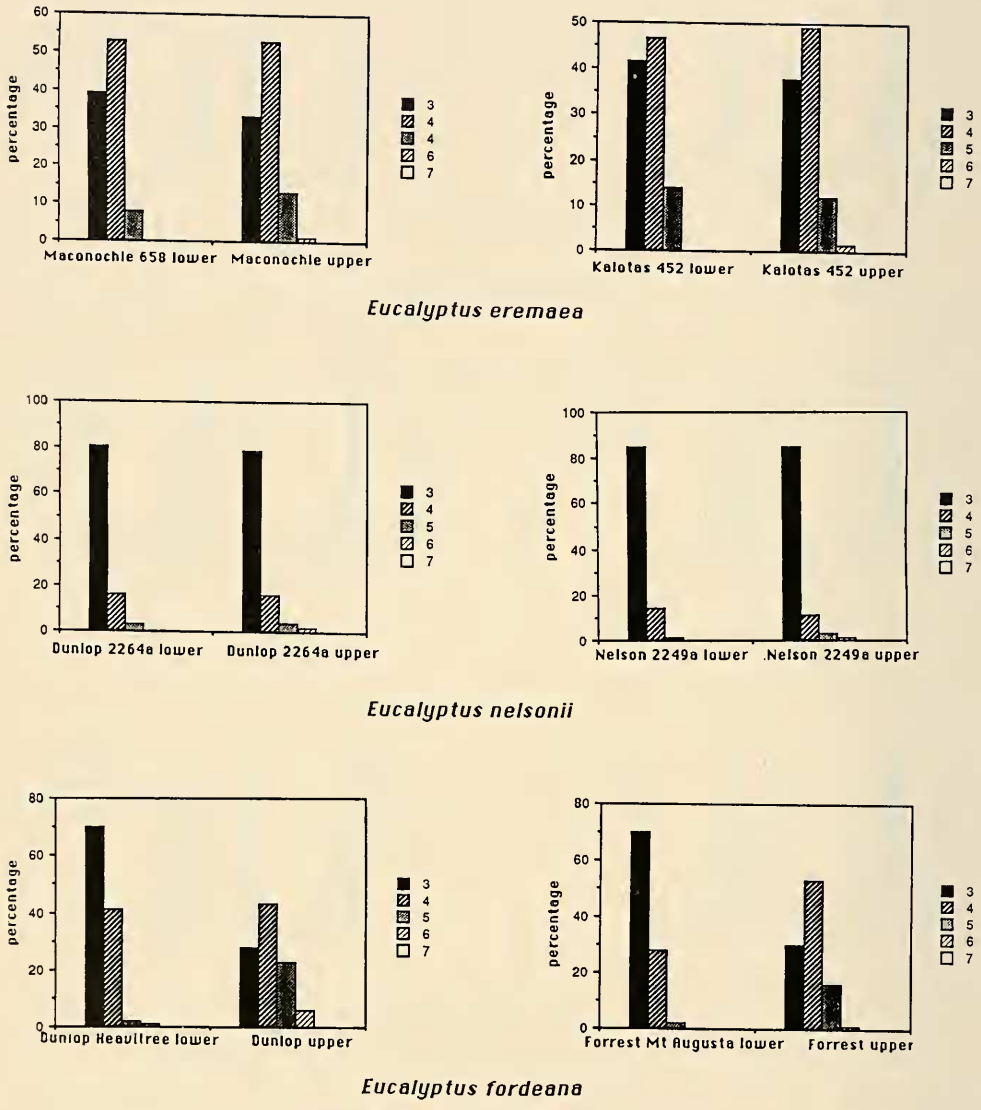


Fig. 26. Frequency spectra of *E. eremaea*, *E. nelsonii* and *E. fordeana*.

In northwestern Western Australia, specimens of two species with somewhat overlapping distributions, *E. pyrophora* and *E. bynoeana* are also readily separable by their frequency-spectra (Fig. 28). Again, in Queensland, specimens of two species, *E. pocillum* and *E. capricornia*, which occur together in a number of areas have quite different frequency spectra (Fig. 29). It is also to be noted that a Northern Territory specimen of *E. capricornia* (Lazarides 7094) has the same frequency spectra as the Queensland

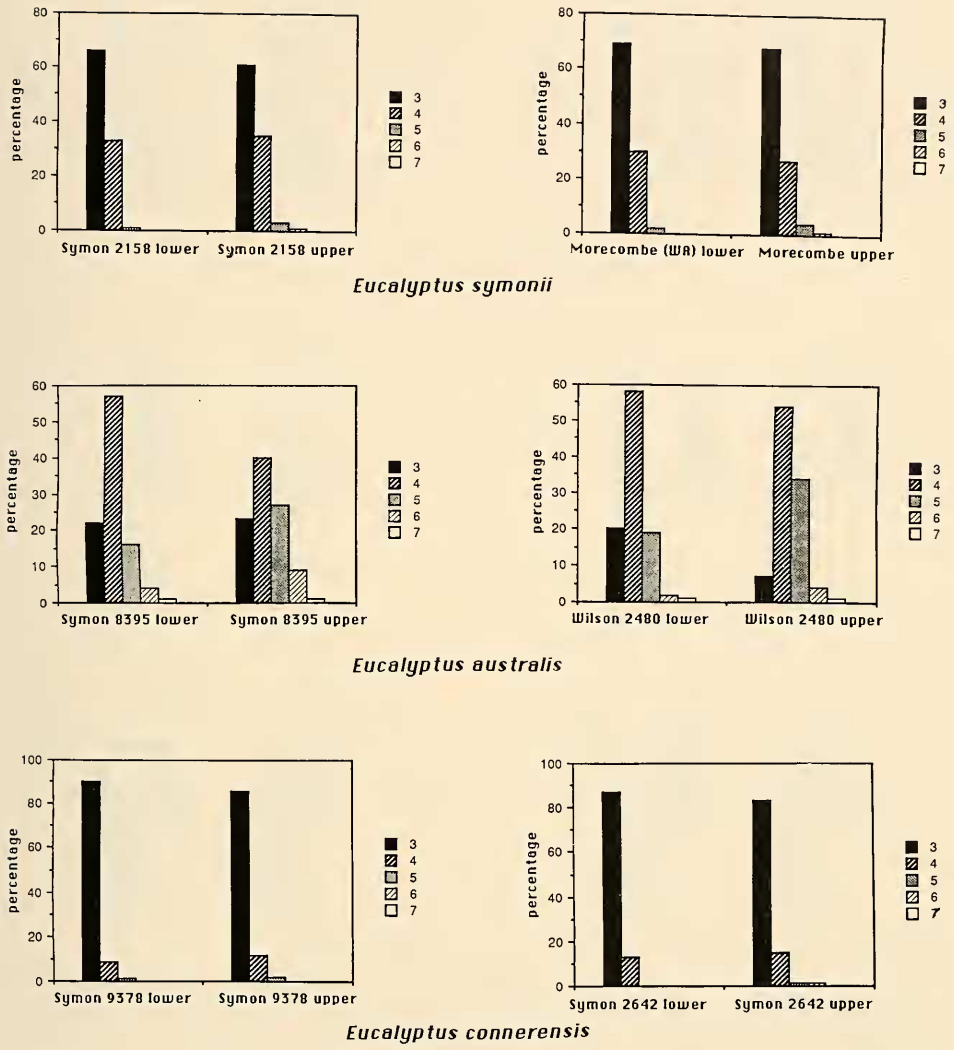


Fig. 27. Frequency spectra of *E. symonii*, *E. australis* and *E. connerensis*.

specimens. This again confirms that, in general, the frequency spectra of widely disjunct specimens of a given species match each other. These quantitative aspects of the phytoglyph are thus of great help in grouping widely disjunct specimens of the same species as well as in discriminating between pairs of sympatric species. The frequency-spectra thus constitute an invaluable tool in dealing with species of the *Corymbosae*. Its potential value in dealing with other groups of eucalypts has still not been assessed, but we recently made some preliminary observations which showed that seedlings of *E. paliformis* and *E. fraxinoides* (*Renantherae*) have useful quantitative phytoglyphic differences.

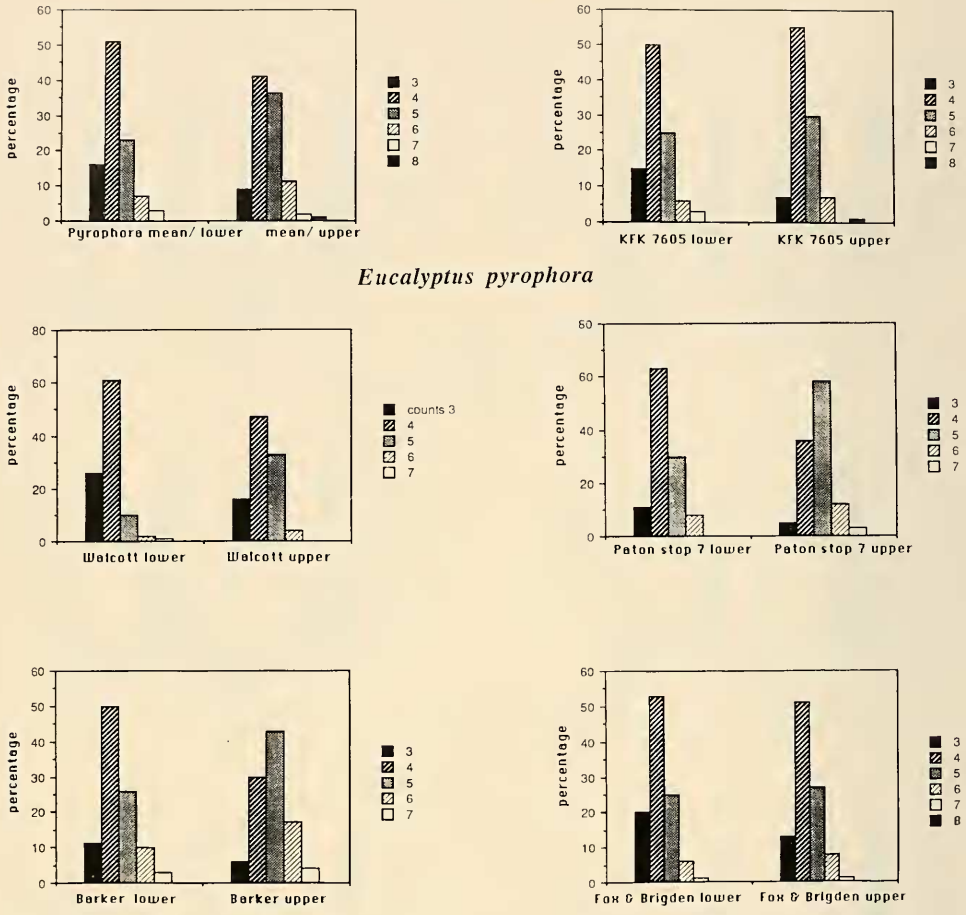
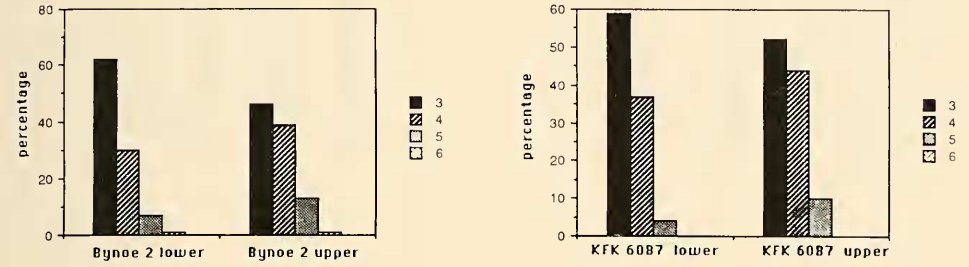


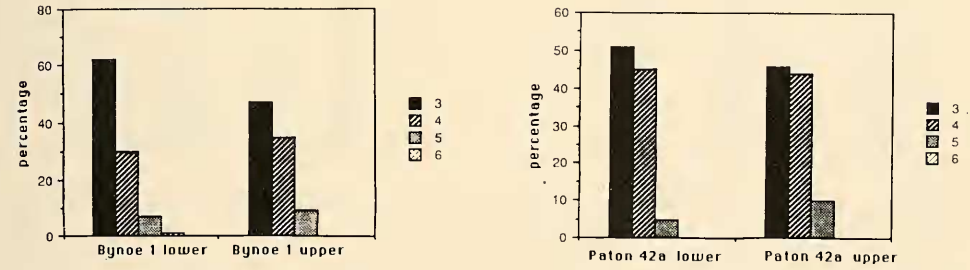
Fig. 28. Frequency spectra of (**1**) *E. pyrophora*, (**2**) *E. bynoeana*.

Consideration of frequency-spectra in terms of development

The basal number of subsidiary cells in the anisocytic stomata of the *Corymbosae* is three. We may consider that higher numbers arise from segmentation of the original 3 subsidiaries in the stomatal complex (Fig. 30). There are two possibilities for division of these initial cells. If each divides once, radially, the stoma will have 6 subsidiary cells; if only one, or only two, divide radially the number will be 4 or 5. Alternatively, one or more subsidiary cells may divide radially more than once, to give 7, 8 or 9 subsidiary cells. This is evidently more likely to occur during the development of giant stomata. The second possibility is that the proto-subsidiary cells divide, not radially, but peripherally. This would leave the subsidiary cell complement at its original number, *if we consider the subsidiary cells as those which have a portion of cell-wall in common with one or both of the guard cells.* In the Commelinaceae, for instance, the stoma may be surrounded by a series of rings of cells, forming a rosette which develops by successive peripheral divisions of 2 or 4 subsidiary cell initials (Tomlinson, 1969). We would use the term subsidiary cells for the innermost cells, those which actually share a wall with the guard cells. In connection with such cases, Napp-Zinn (1973) discusses the problem of

Eucalyptus bynoeana





definition of the term 'subsidiary cell', pointing out that many authors use the term not only for the innermost cells, as defined above, but also for what he terms 'encircling cells' (*Kranzzelle*) which may or may not owe their developmental origin to the single stomatal initial from which the rest of the stomatal complex is derived. Korn's studies of stomata were directed, not to the numbers of subsidiary cells, but to the spacing and pattern of distribution of stomata in the epidermis. Discussing the helicocytic (sequentially spiral) divisions of the stomatal initials of *Sedum stahlii* (Crassulacaeae), the last division of which produces the guard cells, surrounded by (in our definition) 3 or 4 subsidiary cells but, according to Korn, 6 subsidiary cells, Korn (1972) states that: 'Regardless of which cells are true subsidiary cells, the methods employed in models discussed here used cell distances from a developmental aspect and not from the final position of cells. It can be suggested here that perhaps subsidiary cells of *Sedum stahlii* are not subsidiary cells in the traditional sense but may serve as spacers to give ordered arrangements of stomata'. Whether or not the outer rings of cells in cyclocytic stomata, such as those of some genera of Combretaceae (Stace, 1963) and Myrtaceae (Bandulska, 1928-1931) have a

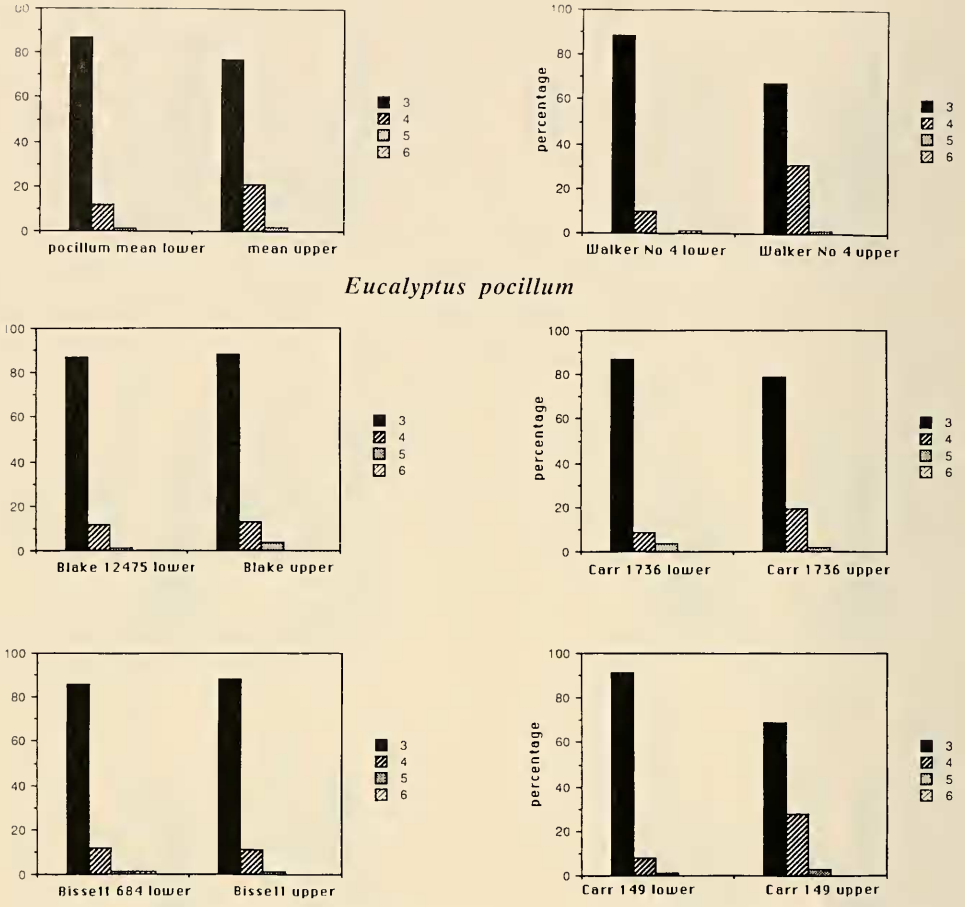
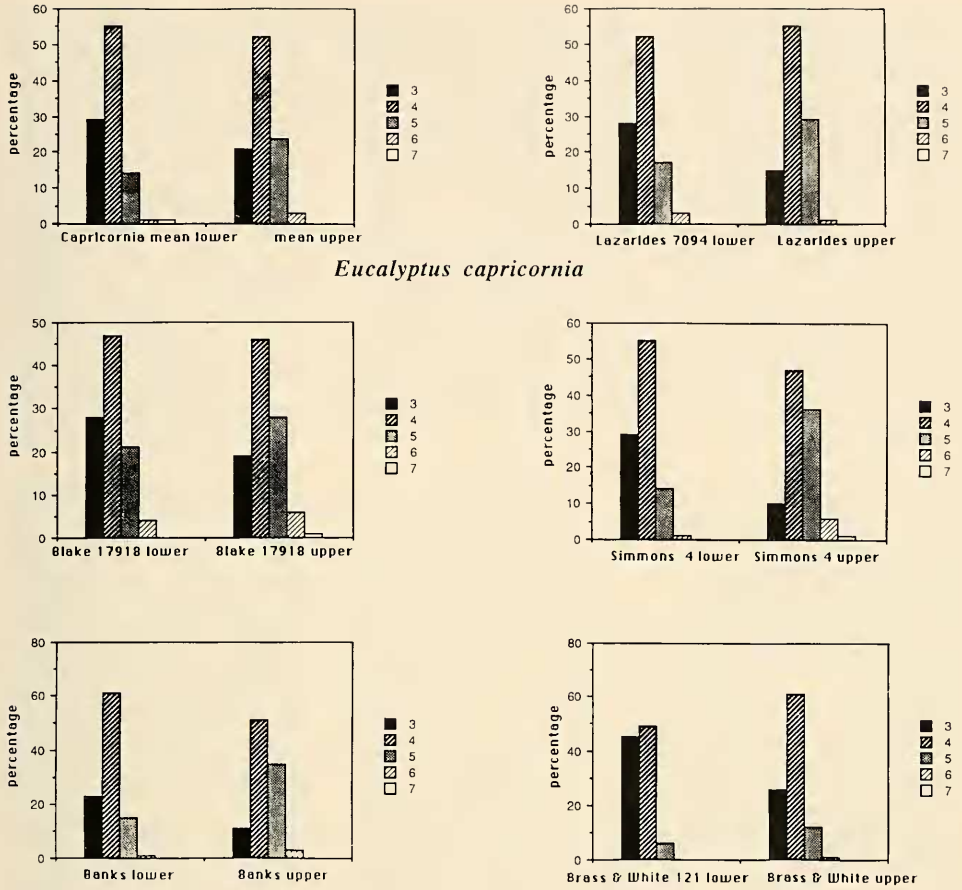


Fig. 29. Frequency spectra of (1) *E. pocillum*, (2) *E. capricornia*. Lazarides 7094 is a specimen of the latter from Northern Territory; the others are from Queensland.

physiological function in stomatal opening and closing like that of the innermost cells is, of course, unknown.

Evidently the species-specific frequency-spectra of the *Corymbosae* must be genetically determined. This must also be the case for the rather rigid, almost mechanical sequences of divisions in many genera of Monocotyledons. It must be true also of families and genera of Dicotyledons in which the patterns of cell division of the stomatal complexes lead to specific and recognized 'types' of stomata, in which the numbers and arrangement of subsidiary cells, either 2, 3 or 4, are fixed and relatively invariant. Some of these patterns have been recognized as characteristic of whole families of flowering plants, e.g. the 'cruciferous', 'ranunculaceous' and 'rubiaceous' types. Because of their recurrence in many families, not only those after which they were named, these types are now classified more objectively in terms of the number and arrangement of the subsidiary cells in the stomatal complexes. A considerable number of 'stomatal types' have been recognized (van Cotthem, 1970; Fryns-Claessens and van Cotthem, 1973; Dilcher, 1974; Wilkinson, 1979). Such classifications have led to the recognition of the widespread distribution of particular 'stomatal types'. For instance, the Magnoliales,



sensu Takhtajan, have a nearly uniform occurrence of paracytic (i.e. 'rubiaceous') stomata, held by some writers to be a primitive type. In the Piperaceae, for instance, the stomata have been described as helicocytic and cyclocytic (van Cotthem, 1971). These relatively 'standard' patterns and numbers must be the products of sets of developmental steps, themselves the resultants of genetic programming by a relatively invariant segment of the genome which is widely distributed in the species and genera of a number of families. This genetic programme determines the pattern and number of divisions giving rise to the stomatal complexes and therefore determines the number of subsidiary cells. Evidently the execution of the developmental steps involved may be subject to occasional aberration but, by and large, the genetically-determined end product will be predominant.

In the *Corymbosae*, the genetic control of the frequency-spectra must also operate by determining the number and orientation of the cell divisions in the cell complexes of the stomatal initials, which are basically anisocytic. We may speculate that the cells, other than guard cells, of the complex have an initial capacity for cell division which is, within certain limits, determined genetically so that there is a statistical probability of a certain mean number of cell divisions, with less probability of fewer or more divisions. Moreover, the general orientation of these cell divisions, either predominantly radial, as in *E. zygophylla* and the related *E. deserticola*, or predominantly peripheral, as in

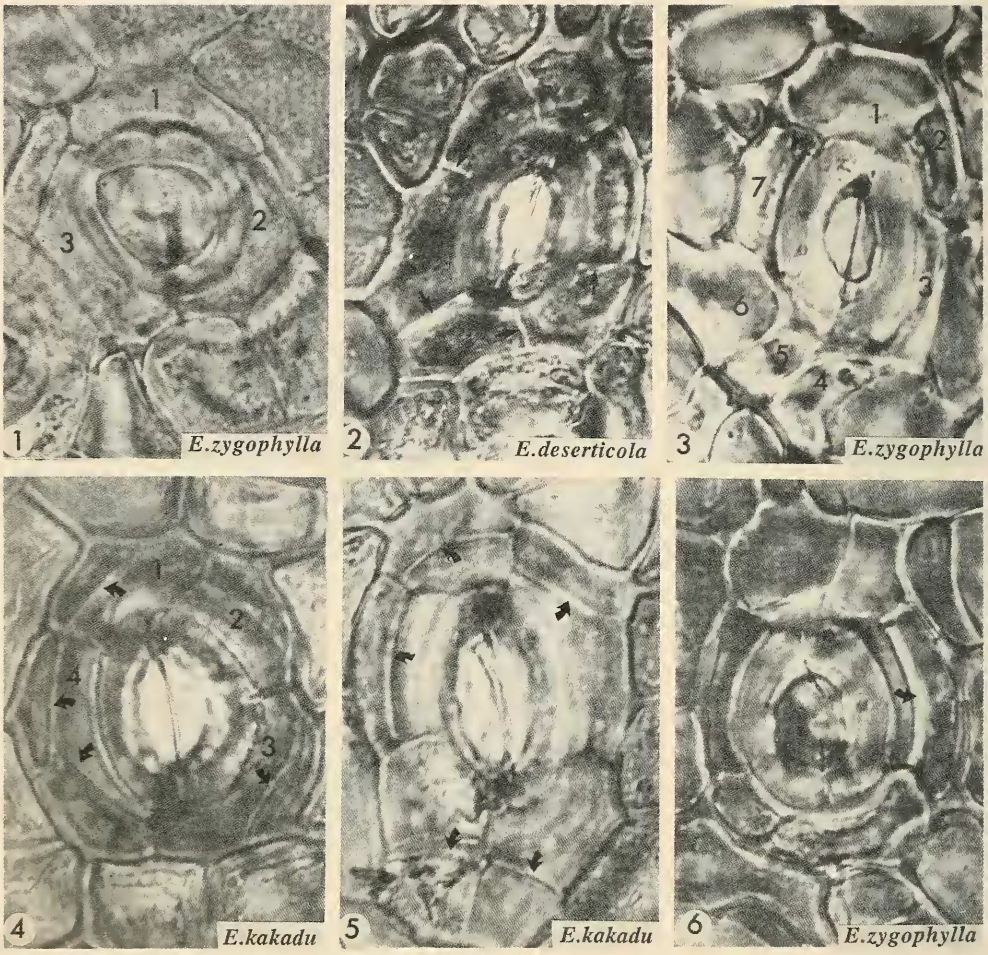


Fig. 30. Stomata to show radial subdivisions (**1-3**) and peripheral subdivision (**4-6**) of the initial subsidiary cells. All to the same scale. Scale bar: 50μm.

E. kakadu, must also be subject to genetic control. One interesting corollary of this view is that there must be genetic determinants which result in the patterns of distribution and of cell division in the stomatal complexes being, in general, different on the lower and the upper surfaces of the leaf.

The differences between Monocotyledons, with their almost mechanical, *deterministically-controlled* patterns of cell divisions in the stomatal complexes and the Dicotyledons, many families and genera of which appear at first glance to have patterns of cell-division which are much more *statistically-determined*, is paralleled by the developmentally-determined orientation of the guard cells in the Monocotyledons and the somewhat random arrangements in many (but not all) Dicotyledons.

References

BANDULSKA, H., 1923. — A preliminary paper on the cuticular structure of certain Dicotyledonous and Coniferous leaves from the Middle Eocene of Bournemouth. *J. Linn. Soc. Lond. Bot.* 46: 241-269.

——, 1928/31. — On the cuticles of some recent and fossil Myrtaceae. *J. Linn. Soc. Lond. Bot.* 48: 657-671.

BARLOW, P. W., and CARR, D. J., (eds), 1984. — *Positional Controls in Plant Development.* Cambridge: Cambridge University Press.

BARTHLOTT, W., and EHLER, N., 1977. — Raster Elektronenmikroskopie der Epidermis-Oberflächen von Spermatophyten. *Tropische und Subtropische Pflanzenwelt.* 19, Mainz.Akad.Wiss.u.d.Literatur. Wiesbaden: Steiner.

BLAKE, S. T., 1953. — Botanical contributions of the Northern Australia Survey. I. Studies on Northern Australian species of *Eucalyptus. Aust. J. Bot.* 1: 185-352.

CARR, D. J., and CARR, S. G. M., 1978. — Origin and development of stomatal microanatomy in two species of *Eucalyptus. Protoplasma* 96: 127-148.

——, and ——, 1980a. — *Eucalyptus* stomata with occluded anterior chambers. *Protoplasma* 104: 239-251.

——, and ——, 1980c. — The *Lehmannianae:* a natural group of Western Australian eucalypts. *Aust. J. Bot.* 31: 629-643.

——, and ——, 1985. — *Eucalyptus I. New or little-known species of the Corymbosae.* Canberra: Phytoglyph Press.

——, and ——, 1987. — *Eucalyptus II. The rubber cuticle and other studies of the Corymbosae.* Canberra: Phytoglyph Press.

CARR, S. G. M., 1972. — Problems of the geography of the tropical eucalypts. *In* D. WALKER, (ed.), *Bridge and Barrier; the natural and cultural history of Torres Strait*: 153-181. Canberra: A.N.U., Dept Biogeogr. and Geomorph. Publ. BG/3.

——, and CARR, D. J., 1976. — Identification of a eucalypt fragment, based on anatomy of leaf and stem. *Proc. Roy. Soc. Vict.* 88: 77-82.

——, and ——, 1979. — An unusual feature of stomatal anatomy in certain taxonomically-related *Eucalyptus* spp. *Ann. Bot.* 44: 230-243.

——, MILKOVITS, L., and CARR, D. J., 1971. — Eucalypt phytoglyphs: the microanatomical feature of the epidermis in relation to taxonomy. *Aust. J. Bot.* 19: 173-190.

CUTLER, D. F., ALVIN, K. L., and PRICE, C E., (eds), 1982. — *The Plant Cuticle.* Linn. Soc. London, Symp. No. 10. London: Academic Press.

DILCHER, D., 1974. — Approaches to the identification of angiosperm leaf remains. *Bot. Rev.* 40: 1-157.

FRYNS-CLAESSENS, E., and VAN COTTHEM, W., 1973. — A new classification of the ontogenetic types of stomata. *Bot. Rev.* 39: 71-138.

KORN, R. W., 1972. — Arrangement of stomata on the leaves of *Pelargonium zonale* and *Sedum stahlii. Ann. Bot.* 36: 325-333.

MAIDEN, J. H., 1909-1928. — A critical Revision of the Genus Eucalyptus. Vols I-VIII. Sydney: Government Printer.

MARTIN, H. A., and JUNIPER, B. E., 1970. — *The Cuticles of Plants.* London: Edward Arnold Ltd.

METCALFE, C. R., 1983. — Secretory structures: Cells, cavities and canals. *In* C. R. METCALFE and L. CHALK, (eds), *Anatomy of the Dicotyledons.* 2nd edition, II: 64-69. Oxford: Clarendon Press.

MUELLER, F. VON, 1874. — *Observations on new vegetable Fossils of the auriferous Drifts.* 2nd decade. Geological Survey of Victoria. Melbourne: Government Printer.

——, 1879-1884. — *Eucalyptographia, a descriptive Atlas of the Eucalypts of Australia etc.* Melbourne: Government Printer.

NAPP-ZINN, K., 1973. — Anatomie des Blattes. II. Angiospermen, A.I. *Encyclopaedia of Plant Anatomy*, viii (2A). Berlin, Stuttgart: Gebrüder Borntraeger.

NEVILLE, A. C., 1963. — Daily growth layers in locust rubber-like cuticle influenced by an external rhythm. *J. Ins. Physiol.* 9: 177-186.

SITHOLEY, R. V., and PANDEY, Y. N., 1971. — Giant stomata. *Ann. Bot.* 35: 641-642.

SMITH, H. G., 1908. — On the elastic substance occurring in the shoots and young leaves of *Eucalyptus corymbosa* and some species of *Angophora. J. Proc. Roy. Soc. N.S.W.* 42: 133-146.

SOLEREDER, H., 1908. — *Systematic Anatomy of the Dicotyledons.* Transl. L. A. BOODLE and F. E. FRITSCH: revised D. H. SCOTT. Oxford: Clarendon Press.

STACE, C. A., 1965. — Cuticular patterns as an aid to plant taxonomy. *Bull. Brit. Mus. (N.H.) Bot.* 4: 1-78.

TOMLINSON, P. B., 1969. — *Anatomy of the Monocotyledons.* III. *Commelinales-Zingiberales.* Oxford: Clarendon Press.

VAN COTTHEM, W. R. J., 1970. — A classification of stomatal types. *Bot. J. Linn. Soc. Lond.* 63: 235-246.

——, 1971. — Vergleichende morphologische Studien über Stomata und eine neue Klassifikation ihrer Typen. *Ber. dtsch bot. Ges.* 84: 141-168.

VAN WYCK, A. E., ROBBERTSE, P. J., and KOK, P. D. F., 1982. — The genus *Eugenia* (Myrtaceae) in southern Africa: the structure and taxonomic value of stomata. *Bot. J. Linn. Soc. Lond.* 84: 411-56.

WELCH, M. B., 1923. — The secretory epidermal cells of certain eucalypts and angophoras. *J. Proc. Roy. Soc. N.S.W.* 57: 218-228.

WILKINSON, H. P., 1979. — The plant surface (mainly leaf). Part I Stomata. *In* C. R. METCALFE and L. CHALK, (eds), *Anatomy of the Dicotyledons.* 2nd edition, I: 97-117. Oxford: Clarendon Press.

ADDENDUM

After delivery of the lecture, the president of the Society, Dr Peter Martin, kindly supplied us with copies of pages of two books on organic chemistry by Professor John Read F.R.S. in which reference is made to Henry G. Smith's discovery of the rubber cuticles of certain bloodwoods and species of *Angophora*. The works are: *A Textbook of Organic Chemistry. Historical, Structural and Economic*, 3rd ed., London: G. Bell & Sons Ltd, 1948, p. 615, and *A Direct Entry to Organic Chemistry*, London: Methuen & Co. Ltd (Home Study Books), 1948 reprinted 1953, p. 227.